ANNUAL RESEARCH PROGRESS REPORT

(FY 2003)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

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MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH

MANAGEMENT UNIT

Project Number: 5450-51000-033-00D **Accession:** 0405041 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: MINERAL ELEMENT REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
Basic research and epidemiologic studies have indicated that inadequate dietary intakes of calcium, copper, magnesium, selenium and excess of iron are associated with altered function of the heart and circulation. Despite these observations, insufficient evidence of threshold dietary levels and mechanisms exist to support scientifically-based dietary recommendations for these elements that will assure cardiovascular health and well-being. To fill this knowledge gap the following approaches are used:
Studies are performed to determine whether intakes of mineral elements (Cu, Mg,) by laboratory animals consistent with mineral intakes of humans are adequate to support optimal cardiovascular function. The focus will be on mineral elements that are known to affect cardiovascular function through oxidative stress and/or nitric oxide-dependent mechanisms. Specific aims include determination of: 1) the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by copper deficiency, and the dietary intakes at which this pathology occurs; 2) whether low copper intakes consistent with that consumed by humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation; 3) whether the oxidative stress induced by copper deficiency affects homocysteine metabolism, and thereby cardiovascular function, by influences upon nitric oxide-dependent signal transduction and mechanisms that promote or protect against atherosclerosis; 4) whether copper status may play a role in metabolic syndrome; 5) whether the consumption of pulses can benefit cardiovascular function be virtue of their high mineral (Cu, Mg) content.
By use of measurements previously confirmed in animal studies, studies are done that examine whether selected minerals (Cu, Mg, Mn, Zn) play roles in human cardiovascular health, and specifically whether indicators of mineral status correlate with mechanistic and functional biomarkers of cardiovascular health. Specific aims are to determine whether: 1) copper supplementation can benefit cardiovascular function in free-living humans; 2) low intakes of magnesium in diets providing high amounts of manganese induce changes in oxidative stress, inflammatory response and neurogenic function to produce cardiovascular pathology; and 3) low dietary zinc influences cardiovascular function through changing oxidative metabolism and copper utilization.

2. **How serious is the problem? Why does it matter?**
Cardiovascular disease is the leading cause of death in this country, with an annual economic impact approaching $180 billion in 2001. Basic research and epidemiological studies have indicated that inadequate dietary intakes of mineral elements such as calcium, copper, magnesium, zinc, nickel, selenium as well as overload of iron are associated with altered functions of the heart and circulation.
Furthermore, dietary surveys indicate that intakes of some of these minerals are not optimal. However, we have little clear, definitive proof that changing dietary practices with regard to these minerals will benefit cardiovascular health in

humans. A clear understanding of how these minerals contribute to cardiovascular function will provide the basis for dietary recommendations that improve the health of the general public.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
Research will address components of National Program 107, Human Nutrition (100%). From component 1, Nutrition Requirements, objectives A (Biomarkers), B (Mechanism of Action), C (Nutrient Interactions), E (Genetic Variability) and G (Function and Performance) will be addressed. From component 2, Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, objective A (Identify nutritional, environmental and genetic factors that modify the effects of nutrient intake and metabolism on health outcomes) will be addressed.

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment during FY 2003:
Although many contractile, electrical and metabolic abnormalities have been found in hearts of copper-deficient animals, characteristics that unequivocally indicate heart failure have not been demonstrated. Scientists at the Grand Forks Human Nutrition Research Center, in collaboration with the laboratory of Y.J. Kang at the University of Louisville, measured variables in hearts of copper-deficient mice that are used in humans to demonstrate heart failure. Hearts of copper-deficient mice were found to have reduced maximum contractile pressure, elevated pressure during relaxation, reduced responsiveness to excitation by adrenalin and increased collagen deposits, all of which are signs of heart failure. IMPACT: This suggests that dietary copper deficiency is a risk factor for heart disease and subsequent cardiac failure.

B. Other significant accomplishments:
Women of child-bearing age consume less than the recommended daily requirement of copper, but it is not known if low dietary copper intake during pregnancy has long-term effects on the cardiovascular system of children. To test this possibility in laboratory animals, bred rats to bear and nurse offspring during maternal copper deficiency; pups were then fed copper-adequate diets for nine months following weaning and assessed for altered heart enzymes and oxidative stress. We found that, despite nine months of copper repletion, rats of copper-deficient dams exhibited an abnormally low activity of cytochrome c oxidase, a copper-dependent enzyme, in heart mitochondria and an increase in heart mitochondrial hydrogen peroxide production. IMPACT: This suggests that damage caused by oxidants generated by cardiac mitochondria could increase the risk of heart disease in progeny of moderately copper-deficient mothers.

In vitro and toxicity studies indicate that responses to dietary nickel can be altered by magnesium status. Urine was collected at 8 and 12 weeks and blood at 13 weeks after weanling rats were placed on diets containing marginally deficient or adequate magnesium, and containing low or physiologically normal amounts of nickel, for the determination of variables associated with cardiovascular health. Low dietary nickel increased the urinary excretion of prostaglandin E-2 and sodium, and the plasma concentrations of triglycerides and glucose; marginal magnesium deficiency enhanced the changes in urinary variables. IMPACT: The findings suggest that nickel and magnesium interact at the kidney level in a manner that can affect blood pressure, that low dietary nickel causes lipid changes undesirable for heart health, and that consuming foods such as leafy green vegetables, legumes, whole grains and nuts, is beneficial to cardiovascular health partly because they provide goodly amounts of nickel and magnesium.

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Recent studies indicate that the occurrences of coronary heart disease and osteoporosis may be correlated. Because dietary copper deficiency has been associated with both diseases, we tested the possibility that copper deficiency could cause both diseases by feeding mice a meat diet (low copper) or a meat plus liver diet (high copper). The low copper diet caused hypercholesterolemia, which is associated with heart disease, and bones with reduced breaking strength. IMPACT: This study of copper deficiency provides a mechanistic explanation of why people with coronary heart disease have excessive osteoporosis and vice versa.

C. Significant accomplishments/activities that support special target populations.
None.

D. Progress Report.
N/A

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
Physiological evidence of a requirement for magnesium by healthy persons has not previously been shown. Postmenopausal women subjects were fed diets that contained 130 mg of magnesium daily or an amount (411 mg daily) greater than the recommended dietary allowance for six months, during which electrocardiographic measurements were made. Subjects consuming the lower amount of dietary magnesium had lower magnesium in red blood cells and in blood serum and a greater number of increased abnormal heart beats compared to those consuming the higher amount of magnesium. IMPACT: These findings illustrate by use of physiological criteria that the magnesium requirement for postmenopausal women exceeds 130 mg daily.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
FY2004 - A study will be completed that will determine whether aberrant carbohydrate metabolism (nonenzymatic glycosylation)is associated with defects of dietary copper deficiency. Numerous delays related to instrumentation and personnel have pushed completion of this project forward from 2003.
A series of studies will be completed that delineate whether elevated cardiac nitric oxide status plays a role in the altered energy metabolism observed in hearts of copper-deficient rats. Unanticipated findings and problems with methods development have delayed completion from 2003.
A series of studies will be completed that will determine the role of insulin-like growth factor (IGF-1) and other growth factors in the altered heart function of copper-deficient rats. Exciting findings have required additional experimentation prior to publication.
A long-term (18-month) study initiated in 2002-03 that is aimed at examining the effect of marginal copper deficiency on parameters related to heart failure (cell death, impaired heart function) in adult rats will be completed. This will extend examination of variables already known to change with severe copper deficiency in growing rats.
Groundwork will be laid with commodities producers that is aimed at examining the efficacy of copper-containing foods in ameliorating the defects of dietary copper deficiency in laboratory animals. Studies will be initiated that will determine the appropriate copper-containing dry edible bean to use in copper supplementation trials for animal and human studies.
Examination of the effects of maternal copper deficiency on the production of reactive oxygen species by heart mitochondria in neonates will continue. Methodology will be developed to determine if the altered cellular redox state increases oxidative carbonylation and nitration of mitochondrial and extra-mitochondrial proteins in heart tissue and oxidative modifications in the DNA of cardiac mitochondria.
An experiment with animals will be performed that will include the determination of

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whether dietary boron influences the beneficial effects of the long-chain omega-3 fatty acids on cardiovascular health.

A series of studies will be initiated that examine the effect of dietary copper deficiency on homocysteine metabolism in laboratory animals.

Low success in recruitment and retention led to insufficient data collection in the human study to determine the excessive dose of zinc supplement at a defined copper intake. The project will continue with improved methods of recruitment.

Animal experiments will continue to identify new indicators of copper status. This year we will study the enzyme paraoxanase because the literature indicates that it is a leading defender against oxidative stress.

Copper deficiency is the only nutritional insult that produces cardiovascular disease AND bone disease in experimental animals. We will begin experiments with animals to clarify the epidemiologic association between ischemic heart disease in osteoporosis. Dietary copper and measurements of oxidative defense will be prominent.

Chelation therapy of ischemic heart disease is being evaluated in a $30 million trial supported by NIH. We will develop a companion grant to evaluate the effect of the treatments on trace element metabolism.

A protocol will be developed to test the hypothesis and that copper supplementation will decrease in frequency of idiopathic ventricular premature discharges in patients with otherwise normal heart physiology.

A study will be completed to identify proteins induced in hearts of copper-deficient rats.

FY2005 - A series of studies will be completed that examine how various isoforms of nitric oxide synthase are altered in hearts of copper-deficient rats and may thus contribute to altered function.

Studies will be initiated that will clarify molecular signaling pathways leading to depressed heart functioning and heart failure. The first pathway to be studied revolves around p38 mitogen activated protein kinase because it is activated by oxidative stress and causes programmed cell death.

Experiments will be initiated that examine the role of nitric oxide in mediating blood pressure changes in copper-deficient rats.

Studies will be initiated that examine dry edible beans as sources of Cu in rats.

In order to correlate oxidative damage caused by copper deficiency with biochemical perturbations in cardiac function, oxidatively modified proteins will be identified and associated changes in the biological functions of the modified proteins will be characterized. This will include alterations in signaling pathways caused by nitrosylation of protein targets phosphorylated by tyrosine kinases.

A human experiment will be completed that ascertains the effect of magnesium deprivation on measures of cardiovascular function including heart rhythm, reactive oxygen species concentrations in blood and urine, blood lipids and excitotoxic neuropeptides in blood.

Studies examining the effect of copper deficiency on homocysteine metabolism in experimental animals will continue.

Animal experiments will continue to identify new indicators of copper status. This year we will study biliverdin reductase because the literature indicates that production of bilirubin by this enzyme acts in defense against oxidative stress, and is protective against atherosclerosis and heart disease.

A protocol will be developed to measure the maternal loss of copper during human pregnancy and to relate trace element measurements on placenta to maternal cholesterol metabolism and infant size; data collection will begin.

FY2006 - Studies will be initiated that examine the role of nitric oxide in altered heart contractile function in copper-deficient animals.

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Studies will continue regarding the effect of dry edible beans on various cardiovascular functions in animals.

Investigations will be conducted to determine whether oxygen radicals generated within heart mitochondria during copper deficiency can cause progressive heart pathology through permanent mitochondrial dysfunction caused by the induction of mutations in mitochondrial DNA. It will be determined whether different types of dietary carbohydrates, lipids, and antioxidants can potentiate or ameliorate oxidative damage in the heart caused by copper deficiency.

Data collection on the previously described (2005) protocol that examines maternal copper loss during pregnancy will be completed.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

F.H. Nielsen made an invited presentation, entitled "Impact of nutrition on the consequences of excessive and inadequate intakes of arsenic," at the Sixth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, Canada, September, 2002.

L.M. Klevay answered several inquiries by individual citizens about copper deficiency and supplements, urinary copper, fatty acid nomenclature, Wilson's disease, nutrient requirements, mercury in milk and food iodine.

L.M. Klevay presented "Hair as a biopsy material in assessing nutriture and intoxication" at the 11th International Symposium on Trace Elements in Man Animals in Berkeley CA.

L.M. Klevay wrote letters to editors pointing out the possible role of copper in findings of beneficial effects of legumes (Archives of Internal Medicine) and of chocolate (American Journal of Clinical Nutrition).

J.T. Saari participated in the conference, "Dry Beans and Health: Research Needs and Possibilities," held at the Grand Forks Human Nutrition Research Center, Grand Forks, ND, July, 2003. The conference was sponsored by the Northarvest Bean Growers Association and included bean growers, members of industry, scientists and policy makers interested in clarifying the scientific basis for the health benefits of dry edible beans. Discussions among participants are ongoing.

J.T. Saari presented the research goals and accomplishments of the Management Unit on Mineral Nutrients for Optimal Function and Health to scientists from Zinpro, a company providing mineral supplements to the livestock industry, July, 2003.

W.T. Johnson presented "Copper deficiency and oxidative pathways in cardiovascular disease" for the Faculty Lecture Series, University of North Dakota School of Medical and Health Sciences, Grand Forks, North Dakota, September, 2003.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**

L.M. Klevay was a delegate to the Healthy North Dakota Summit in Bismarck ND.

W.T. Johnson wrote an article in the nutrition section of The Grand Forks Herald titled ¿Eating less-The new fountain of youth.¿

Publications: **Log 115:**

1. Schuschke, D.A., Percival, S.S., Lominadze, D., Saari, J.T., Lentsch, A.B. Tissue-specific ICAM-1 expression and neutrophil transmigration in the copper-deficient rat. Inflammation. 2002. v. 26. p. 297-303. **0000135448**
2. Elsherif, L., Ortines, R.V., Saari, J.T., Kang, Y.J. Congestive heart failure **0000142014**

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in copper deficient mice. Experimental Biology and Medicine. 2003. v. 228. p. 811-817.

3. Hintz, K.K., Relling, D.P., Saari, J.T., Borgerding, A.J., Duan, J., Norby, F.L., Ren, B.H., Kato, K., Epstein, P.N., Ren, J. Cardiac over-expression of alcohol dehydrogenase exacerbates cardiac contractile dysfunction, lipid peroxidation and protein damage following chronic ethanol ingestion. Alcoholism: Clinical and Experimental Research. 2003. v. 27. p 1090-1098. **0000143351**

4. Folden, D.V., Gupta, A., Sharma, A.C., Li, S-Y., Saari, J.T., Ren, J. Malondialdehyde inhibits cardiac contractile function in ventricular myocytes via a p38 mitogen-activated protein kinase-dependent mechanism. British Journal of Pharmacology. 2003. v. 139. p. 1310-1316. **0000147697**

5. Yokoi, K., Uthus, E.O., Nielsen, F.H. Nickel deficiency diminishes sperm quantity and movement which possibly impairs reproductive function in rats. Biological Trace Element Research. 2003. v. 93. p. 141-153. **0000127981**

6. Milne, D.B., Nielsen, F.H. High dietary fructose compared with corn starch does not heighten changes in copper absorption, retention, or status indicators in men fed low dietary copper. Journal of Trace Elements in Experimental Medicine. 2003. v. 16. p. 27-38. **0000128423**

7. Nielsen, F.H., Milne, D.B. Some magnesium status indicators and oxidative metabolism responses to low-dietary magnesium are affected by dietary copper in postmenopausal women. Nutrition. 2003. v.19. p. 617-626. **0000133425**

8. Klevay, L.M., Wildman, R.E.C. Meat diets and fragile bones: Inferences about osteoporosis. Journal of Trace Elements in Medicine and Biology. 2002. v. 16. p. 149-154. **0000123092**

9. Klevay, L.M. Copper in legumes may lower heart disease risk. Archives of Internal Medicine. 2002. v. 162. p. 1780. **0000134436**

10. Klevay, L.M. Extra dietary copper inhibits LDL oxidation. American Journal of Clinical Nutrition. 2002. v. 76. p. 687-688. **0000129912**

11. Elsherif, L., Ortines, R., Saari, J.T., Kang, Y.J. Oxidative stress and heart failure in a mouse model of copper deficiency. FASEB Journal. 2003. v. 17. p. A229. Presented by L. Elsherif at Experimental Biology 2003 meeting. San Diego, CA. April, 2003. **0000148031**

12. Saari, J.T. Differential effects of dietary copper deficiency on respiration via cardiac mitochondrial complexes I and II. FASEB Journal. 2003. v. 17. p. A1128. Presented by J.T. Saari at Experimental Biology 2003 meeting. San Diego, CA. April, 2003. **0000141342**

13. Elsherif, L., Ortines, R., Saari, J.T., Kang, Y.J. Hypertrophy and heart failure in a mouse model of copper deficiency. FASEB Journal. 2003. v. 17. p. A1128. Presented by L. Elsherif at Experimental Biology 2003 meeting. San Diego, CA. April, 2003. **0000148029**

14. Lominadze, D., Saari, J.T., Schuschke, D.A. Neutrophil migration under agarose is altered by copper deficiency. FASEB Journal. 2003. v. 17. p. A1128. Presented by D. Lominadze at Experimental Biology 2003 meeting. San Diego, CA. April, 2003. **0000141978**

15. Johnson, W.T. Changes in respiratory complex activities of heart mitochondria caused by copper deficiency during development in rats are resistant to copper supplementation. FASEB Journal. 2003. V. 17. p. A378. Presented by W.T. Johnson at Experimental Biology 2003 Meeting. San Diego, CA. April, 2003. **0000141290**

16. Nielsen, F.H. Boron deficiency alters platelet aggregation differently than **0000141295**

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replacing palm oil with canola oil in the diet of rats. FASEB Journal. 2003. v. 17: p. A707. Presented by F.H. Nielsen at Experimental Biology 2003 meeting. San Diego, CA. April, 2003.

17. Johnson, W.T. Oxidative stress resulting from inhibition of the mitochondrial electron transport chain contributes to the induction of hepatic heme oxygenase-1 in copper deficient rats. Journal of Nutrition. 2003. v. 133 (#5S-1) p. 203E. Presented by W.T. Johnson at the 11th International Symposium on Trace Elements in Man and Animals, Berkley, CA, June 2002. 0000131355

Approved: ROOS ERIC E **Date:** 09/26/2003

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GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE AND JOINT DEVELOPMENT AND HEALTH

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

Bone and joint diseases are major causes of illness in the US. For example, one out of every two women and one out of eight men over age 50 will have an osteoporosis-related fracture in their lifetime. However, the causes of osteoporosis and many of the rheumatic diseases (e.g., osteoarthritis and rheumatoid arthritis) are unknown with the notable exception of those determined to have a nutritional (e.g., rickets) or a heritable (e.g., osteogenesis imperfecta) basis. Although it is generally agreed that these diseases (e.g., osteoporosis and arthritis) are of complex etiology with genetic, nutritional and hormonal factors, no effective prevention strategies are known. The treatments available for osteoporosis, osteoarthritis, or rheumatoid arthritis are characterized by either limited therapeutic value or pharmacologic intervention with significant potential for undesirable side effects. Adequate trace element nutriture is a promising but meagerly examined candidate for helping prevent or ameliorate these bone diseases. For example, several recent findings are consistent with the hypothesis that nutritional copper and zinc deficiencies contribute to the occurrence of bone fractures by reducing bone strength in adults. Physiologic amounts of dietary boron correct manifestations of experimental rheumatoid arthritis in animal models, and improve subjective measures of osteoarthritis in volunteers with the disease. Thus, there is ample reason to determine whether acquired adult trace element deficiencies play a role in the pathogenesis or treatment of osteoarthritis, rheumatoid arthritis, and osteoporosis.

Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various ultratrace (including boron, nickel, and silicon), trace (including copper and zinc), and macro (including magnesium and calcium) elements for optimal bone and joint development and health. The basic approach was to feed experimental animals and human volunteers diets that contained low, adequate, and/or supranutritional amounts of specific trace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific trace elements). The experimental designs typically simulated specific characteristics of the target population (e.g., ovariectomized rats are used to simulate the postmenopausal state; an injected antigen in rats simulated rheumatoid arthritis). The response of the animals and humans to the dietary manipulations was ascertained by evaluating appropriate biochemical, physiological, and anatomical variables.

2. **How serious is the problem? Why does it matter?**

It has been estimated that the lifetime risk of fracture exceeds 40% for women and 13% for men. In the elderly, hip fractures are associated with mortality in up to 20% of the cases, with costly long-term nursing home care required for most survivors. Osteoarthritis and osteoporosis alone currently cost more than $50 billion each year in medical costs and lost wages. The annual cost of healthcare related to osteoporosis is estimated at $14 billion. The lifetime cost of rheumatoid

arthritis for an individual patient can be as much as $250,000. As the US population ages, the economic cost of osteoporosis alone is projected to reach $50 billion by the year 2040. In addition to the direct costs, diseases of bones and joints reduce personal independence and quality of life. Dietary modification is a sensible, practical and economically feasible approach to the prevention of osteoporosis and arthritis. As trace minerals are known to be essential for bone health, it is important that we determine their specific role in prevention of these devastating diseases.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**

The research program relates to the National Program 107, Human Nutrition, and emphasizes the Program Component Performance Goal 3.1.1- Human Nutrition Requirements. The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate the detection and prevention of biochemical, structural, physiological and psychological dysfunctions caused by the deficiency or imbalance of specific trace elements, and will define requirements and safe intakes of specific trace elements for health and well-being throughout the life cycle.

4. **What were the most significant accomplishments this past year?**

A. Single Most Significant Accomplishment during FY 2003:

Diabetes mellitus, a disease caused by a deficiency of insulin, results in many severe health problems including osteoporosis. The Grand Forks Human Nutrition Research Center conducted studies with healthy rats to determine whether boron, a natural substance in the diet, helps regulate the level of insulin in the blood. The studies demonstrated that a diet with normal physiological amounts of boron reduced blood insulin levels without affecting glucose levels. Impact: These results suggest that dietary boron, found in higher concentrations in fruits, vegetables, nuts, and legumes, may help reduce the amount of insulin needed to maintain blood glucose and thus protect the insulin-secreting cells from "exhaustion" and development of diabetes mellitus.

B. Other Significant Accomplishment(s):

The concerns about inadequate intakes of calcium and iron have led to extensive fortification of both minerals in the US food supply, but the effects of the interaction of these nutrients on bone health are poorly understood. The Grand Forks Human Nutrition Research Center, in collaboration with Dr. John Wagner at the University of North Dakota, conducted a study with growing female rats to determine whether an interaction between calcium and iron affects the biomechanical and biochemical properties of bone. The findings from this study indicate that higher than adequate calcium intakes reduced both iron status and bone quality. Impact: These findings indicate that very high calcium intakes, as may result from indiscriminate supplementation and fortification of foods with calcium, may compromise the iron status of vulnerable segments of the population without offering additional benefits to bone health.

There is emerging evidence from human and animal experiments that silicon supplementation might be a nutrient that is beneficial to bone formation and maintenance, but the biochemical indicators needed to ascertain such effects in humans have not been established. In an experiment performed at the Grand Forks Human Nutrition Research Center, urine and blood were collected from 3-month-old rats that were fed diets containing low or physiologically normal amounts of silicon

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starting after weaning. Low dietary silicon increased the urinary excretion of two bone-collagen breakdown products and a plasma protein involved in bone turnover. Impact: The changes in these products indicate that they are useful indicators in determining the nutritional effects of silicon on bone health in humans and that silicon has a function that affects bone and joint collagen turnover.

Because dietary nickel and magnesium, two minerals generally supplied by the same foods, affect the metabolism of prostaglandin E-2 which influences bone formation, a combined lack of these minerals possibly could be detrimental to bone health. In an experiment performed at the Grand Forks Human Nutrition Research Center, urine was collected at 8 and 12 weeks, and bones were obtained at 13 weeks after weanling rats were placed on diets either marginally deficient or adequate in magnesium, and containing low or physiologically normal amounts of nickel. The combined marginally deficient magnesium and low nickel diet resulted in increased prostaglandin E-2 excretion and changed the mineral composition of bone, but did not enhance changes in bone breaking characteristics induced by low nickel or magnesium deficiency alone. Impact: The findings indicate that consuming foods high in both magnesium and nickel, including leafy green vegetables, legumes, whole grains and nuts, is beneficial to bone health, and that the beneficial effects possibly occur through these minerals influencing the metabolism of lipids to prostaglandins.

A high meat intake is often cited as a risk factor for development of osteoporosis, and soy protein is thought to have a beneficial effect on bone health. However, new reported findings from a human study at the Grand Forks Human Nutrition Research Center indicate that partial substitution of soy protein (with high isoflavones) for meat protein does not improve calcium retention in postmenopausal women. In a follow-up study with ovariectomized rats, complete substitution of meat protein by soy protein isolate with high isoflavone content reduced calcium retention by about 30%. Impact: Assuming that the findings in rats are applicable to humans, these combined results indicate that, contrary to popular belief, meat protein intake does not adversely affect bones and that while a partial substitution of soy protein for meat protein may not reduce calcium retention, full replacement may adversely affect calcium retention.

Normal physiological amounts of boron are known to be essential or beneficial for the growth and development of several animal models of human nutrition but there is no information on the amount of boron supplied by human milk to the breast-fed infant. The Grand Forks Human Nutrition Research Center, in collaboration with Dr. James Friel at the University of Manitoba, Canada, determined the concentration of boron in human milk from mothers of full-term and premature infants living in St. John's, Newfoundland, Canada. The findings indicate that the average concentration of boron in milk from nursing mothers from this locale is 0.03 milligrams per liter, is not affected by infant prematurity and, in contrast to several other trace minerals, does not fall during the first 12 weeks of lactation. Impact: The findings indicate that, similar to calcium, the concentration of boron in human milk may be regulated.

C. Significant Accomplishments/Activities that Support Special Target Populations.
None.

D. Progress Report.
None.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

The US Food and Drug Administration has approved a health claim that a daily consumption of 25 g of soy protein is beneficial to heart health. However, the effects of this dietary practice on bone health are unknown. A controlled feeding

trial conducted at the Grand Forks Human Nutrition Research Center (GFHNRC) with postmenopausal women, using sensitive whole body counting methodology, demonstrated that when 25 g of soy protein is substituted for an equal weight of meat protein, on a daily basis for 8 weeks, the amount of calcium that the body retains does not change. Furthermore, urinary and blood biomarkers of bone formation and breakdown did not change. Impact: These findings indicate that the common practice of substitution of soy protein for meat protein does not provide any additional benefits (associated with phytoestrogens) or risks (associated with phytate) with regards to calcium retention or bone health in postmenopausal women and that the effects of vegetable proteins versus animal proteins on bone health deserve further investigations.

6. **What do you expect to accomplish, year by year, over the next 3 years?**

FY2004 - The hypothesis that silicon is an essential nutrient involved in the formation or function of bone matrix components including collagen and glycosaminoglycans which, if suboptimal, increases the risk for osteoporosis will be tested by using a SASO-2 cell culture system to ascertain the effect silicon at the molecular level.

An experiment will be performed with experimental animals to determine whether urinary and blood markers of bone turnover are altered by a relationship between boron and the omega-3 fatty acids that could be used as a basis for a human study to ascertain the influence of these dietary substances on the susceptibility or prevention of osteoporosis.

An experiment will be performed in collaboration with the University of Buenos Aries to determine whether silicon status affects the formation of bone around dental and bone implants.

An experiment will be completed that determines whether dietary silicon helps prevent bone loss in an animal model of osteoporosis (ovariectomized adult rats).

The previously initiated three-year, double-blind placebo-controlled supplementation trial will continue to compare the effects of calcium supplementation with and without copper and zinc supplementation on bone loss in postmenopausal women.

Controlled feeding studies designed to test the effects of interaction between meat protein and dietary calcium on calcium retention and bone metabolism will be conducted.

Animal studies investigating the interaction of meat protein and dietary calcium on calcium retention and biomechanical and biochemical properties of bone as well as the IGF-1 proteins will be conducted in ovarioectomized rats.

Studies will be initiated to test the feasibility of use of calcium-41 combined with accelerated mass spectrometry in nutrition research, with emphasis on bone metabolism.

Controlled feeding studies designed to compare calcium retention from soy milk versus dairy milk will be conducted.

Because boron in the diet has many beneficial effects on human and animal physiology, an experiment will be designed and initiated to examine the uptake and retention of boron from a common food source in healthy volunteers.

Boron deprivation is known to affect inflammation in humans and animal models. A study initiated previously will be continued to determine the effects of boron deprivation on manifestations of rheumatoid arthritis in human subjects with an emphasis on measuring variables that will indicate whether low boron nutriture, not uncommon in the United States, is a factor in the prevalence and severity of rheumatoid arthritis.

Studies will be initiated to elucidate the induction mechanism whereby dietary boron increases the concentration of natural killer cells in the serum of animal models with experimental rheumatoid arthritis.

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Animal studies will be conducted to determine why a semi-purified diet based on ground corn and supplemented with boron protects better against experimental arthritis than does a commercial rodent chow with ample boron.

FY2005 - Animal studies investigating the effects and mechanisms of action of trace minerals on the IGF-1 proteins and bone status will be conducted in growing and overarioectomized rats.

Studies will continue to test the feasibility of use of calcium-41 combined with accelerated mass spectrometry for measurement of bone resorption.

Based on animal experiments, a human experiment will be designed that will ascertain whether low silicon intakes are detrimental to maintaining bone density and strength.

Complete a human experiment to determine whether magnesium deficiency induces changes in neuropeptide metabolism or causes neurogenic dysfunction such that it results in bone loss leading to osteoporosis.

Health is impaired when diets are too low in boron, a fact that implies that specific biomolecules require boron. Capillary electrophoresis experiments will be conducted to determine which candidate biomolecules, selected on the basis of their chemical structure, bind tightly to boron.

Because the concentration of diadenosine oligophosphates are higher in platelets than any other tissue compartment and bind tightly to boron, an experiment will be performed with experimental animals to determine whether blood coagulation factors are affected by dietary boron.

To determine how dietary boron affects bone structure, bone and cartilage extracellular matrix microarchitecture, with emphasis on collagen spatial orientation, will be examined in rats fed low or physiological normal amounts of boron.

To determine the mechanism(s) by which dietary boron influences immune cell function, Th1 and Th2 cytokines will be measured in a series of experiments with rats fed low or physiological normal amounts of boron.

FY 2006 - Controlled feeding studies designed to determine the effects of non-digestible carbohydrates on calcium retention and trace mineral status will be conducted.

Controlled feeding studies will be conducted, using calcium-41/accelerated mass spectrometry methodology, to test the effects of graded intakes of calcium on bone resorption with the goal of defining calcium requirements in postmenopausal women.

The human experiment designed in 2005 to ascertain the practical importance of silicon in bone formation and maintenance will be conducted.

An experiment will be performed with experimental animals to determine whether urinary and blood markers of bone turnover are altered by a relationship between nickel content and the fatty acid composition of the diet that could be used to ascertain whether nickel is a practical concern for bone health in humans.

To determine the role of dietary boron in hemostasis, selected enzymes in the clotting cascade in rats will be examined for their response to dietary boron.

Because boron inhibits the activity of numerous serine protease enzymes, a series of experiments with rats will be conducted to determine whether selected proteases modulate T-lymphoid and natural killer cell growth.

Because boron is important in normal bone development, tissue culture experiments will be conducted to identify the class of molecules in the extracellular matrix most likely to require boron for proper matrix synthesis.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

Information about the nutritional of beneficial aspects of ultratrace and trace

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elements as it became available was routinely transferred to a variety of customers. The customers included nutritional risk assessment groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations, via the popular media; and other scientists through presentations at national and international meetings and professional publications.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**

The ARS Daily News Service provided a press release on findings from a recent publication authored by Z.K. Roughead that described the effects of meat protein on calcium metabolism. The release was picked up by various news outlets including the Food Industry Environmental Network.

Information was transferred to the public through interviews on two local television stations, and various presentations to regional groups including the North Dakota Stockmen's Association.

Information was transferred to the public through the local newspaper (Grand Forks Herald) that was also placed on the Grand Forks Human Nutrition Research Center Home Page.

Publications: **Log 115:**

1. Roughead, Z.K., Johnson, L.K., Lykken, G.I., Hunt, J.R. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. Journal of Nutrition. 2003. v.133. p.1020-1026. **0000134479**
2. Roughead, Z.K., Lukaski, H.C. Inadequate copper intake reduces serum insulin-like growth factor-1 and bone strength in growing rats fed graded amounts of copper and zinc. Journal of Nutrition. 2003. v.133. p.422-448. **0000138983**
3. Seaborn, C.D., Nielsen, F.H. Silicon deprivation decreases collagen formation in wounds and bone, and ornithine transaminase enzyme activity in liver. Biological Trace Element Research. 2002. v.89. p.251-261. **0000127551**
4. Seaborn, C.D., Nielsen, F.H. Dietary silicon and arginine affect mineral element composition of rat femur and vertebra. Biological Trace Element Research. 2002. v.89. p.239-250. **0000127586**
5. Ren, J., Hintz, K.K., Roughead, Z.K., Duan, J., Colligan, P.B., Ren, B.H., Lee, K.J., Zeng, H. Impact of estrogen replacement on ventricular myocyte contractile function and protein kinase B/Akt activation. American Journal of Physiology Heart and Circulatory Physiology. 2003. v.284. p.H1800-H1807. **0000145609**
6. Ren, J., Roughead, Z.K., Wold, L.E., Norby, F.L., Rakoczy, S., Mabey, R.L., Brown-Borg, H.M. Increases in insulin-like growth factor-1 level and peroxidative damage after gestational ethanol exposure in rats. Pharmacological Research. 2003. v.47. p.341-347. **0000135856**
7. Hunt, C.D. Boron. Caballero, B., Trugo, L., Finglas, P., (eds). Academic Press,London. Encyclopedia of Food Sciences and Nutrition. 2003. p.566-574. **0000127578**
8. Nielsen, F.H. Silicon nutrition affects urinary and plasma indicators of bone and connective tissue metabolism. Anke, M., Muller, R., Schafer, U., Stoeppler, M., (eds.). Schubert-Verlag, Leipzig, Germany. Macro and Trace Elements (Mengen- und Spurenelemente). 2002. p.1231-1237. Presented by F.H. Nielsen at the Workshop on Macro and Trace Elements, Jena, Germany, October 2002. **0000139340**
9. Roughead, Z.K. Is the interaction between dietary protein and calcium destructive or constructive for bone?: Summary. Journal of Nutrition. 2003. v.133. p.866S-869S. **0000144728**

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10. Hunt, C.D., Idso, J.P. Hyperinsulinemia in rats during dietary vitamin D (VITD) and boron deprivation is ameliorated, but not corrected, by improved VITD status. The FASEB Journal. 2003. v.17. Abstract. p.A707. Presented by C.D. Hunt at the Experimental Biology 2003 meeting. San Diego, CA. April 2003. 0000141276

11. Hunt, C.D. Dietary boron alleviates growth cartilage abnormalities induced by vitamin D deficiency in chicks. 11th International Symposium on Trace Elements in Man and Animals. Journal of Nutrition. 2003. v.133(5S-1). p.230E-231E. 0000131353

12. Combs Jr., G.F., Hassan, N., Hunt, C.D. Risk factors for rickets in Bangladesh. Journal of Trace Elements in Experimental Medicine. 2003. v.16. Abstract. p.114. Presented by G.F. Combs Jr. at the VIth International Society of Trace Element Research in Humans Conference, Quebec City, Canada. September 2002. 0000135642

13. Nielsen, F.H. Silicon deficiency affects urinary indicators of bone breakdown differently in ovariectomized and ovary-intact rats. The FASEB Journal. 2003. v.17. Abstract p.A706. Presented by F.H. Nielsen at the Experimental Biology 2003 meeting. San Diego, CA. April 2003. 0000141296

14. Roughead, Z.K., Lykken, G.I., Johnson, L.K. Substituting soy protein for meat protein did not affect calcium (Ca) retention or biomarkers of bone and cardiovascular health in a controlled feeding study of healthy postmenopausal women. The FASEB Journal. 2003. v.17. Abstract. p.A705. Presented by Z.K. Roughead at the Experimental Biology 2003 meeting. San Diego, CA. April 2003. 0000141857

15. Durick, K., Lamoureux, J., O'Bryant, D., Griffiths, M.M., Hunt, C.D., Bradley, D.S. Accessible dietary boron prevents the onset and down regulates ongoing collagen-induced arthritis. Book of Abstracts for the 2002 International Symposium on Connective Tissue Research. Autoimmune Arthritis: New Directions.Presented by K. Durick at the Symposium in Memphis, TN. October 2002. 0000147862

16. Durick, K., Lamoureux, J., O'Bryant, D., Griffiths, M.M., Hunt, C.D., Bradley, D.S. Dietary boron supplementation effectively mitigates collagen-induced arthritis in mice. Frank N. Low Research Day. Abstract. p.53. Presentation by K. Durick at the 23rd Annual Frank N. Low Research Day, Grand Forks, ND. April 2003. 0000149020

Approved: ROOS ERIC E **Date:** 09/26/2003

Project Number: 5450-51000-034-02T **Accession:** 0407132 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: MEAT PROTEIN AND CALCIUM: DO THEY INTERACT SYNERGISTICALLY OR ANTAGONISTICALLY?

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
The Food and Drug Administration has approved a health claim stating that a daily consumption of 25 g of soy protein, as part of a low fat diet, may reduce the risk of heart disease. Recent studies suggest that soy protein may also protect against postmenopausal bone loss. In contrast, the consumption of meat protein is often cited as a risk factor for osteoporosis. To date, no study has compared the effects of substituting soy protein for meat protein on calcium retention under controlled conditions. Because the consumption of soy protein as a meat substitute is increasing in this country, a careful assessment of the effects of this dietary practice on calcium retention is needed.

2. **How serious is the problem? Why does it matter?**
In the elderly, hip fractures associated with osteoporosis cause mortality in up to 20% of the cases, with costly long-term nursing home care required for most survivors. The notion that meat consumption is a risk factor for the development of osteoporosis and soy protein is protective is not based on observations from controlled studies and deserves further examination. As inadequate protein and zinc status are common nutritional problems in the elderly, meat is an excellent source of both nutrients, and both proteins are commonly consumed, it is important to give evidence-based advice about the risks and benefits of consuming meat and soy proteins on maintenance of bone health and prevention of osteoporosis.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
This research relates to the National Program 107, Human Nutrition. Specifically, it addresses nutrient requirements, mechanisms of action of nutrients, identification of biomarkers (for the study of bone status), nutrient-nutrient and nutrient-gene interactions, identification of health promoting properties of plant and animal foods, and health promoting intervention strategies for targeted populations.

4. **What were the most significant accomplishments this past year?**
This report serves to document research conducted under a Trust Fund Cooperative Agreement, #58-5450-3-41, between ARS and the National Cattlemen's Beef Association. Additional details of research can be found under the report for the parent project 5450-51000-034-00D.

A controlled feeding study of postmenopausal women was completed to determine calcium retention (by using calcium-47 as a tracer and whole body scintillation counting) from diets containing meat protein to those in which 25 grams of soy protein was substituted for meat protein. Fractional calcium absorption was very similar between the two diets indicating that meat consumption does not increase calcium loss from the body and any beneficial effects of soy protein on bone are unrelated to changes in intestinal absorption of calcium and may be related to its utilization by bone. As the effect of soy protein consumption on calcium metabolism is not adequately tested and soy consumption is on the rise, especially in

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postmenopausal women, the findings from this study have important implications for providing evidence-based advice to the public regarding the risks and benefits of consuming soy and meat protein for bone health in the context of a mixed diet.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
Over the life of the project, it is expected that our understanding of the effects of animal protein versus vegetable protein consumption on bone metabolism will be enhanced. It is expected that the data base on the effects of common dietary practices such as consuming soy protein versus meat protein on bone metabolism will be expanded.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
FY 2004: The analysis of data from in this study (15 postmenopausal women) are complete, the results have been reported at major scientific meetings and a manuscript is in preparation for publication in a peer-reviewed journal.

FY 2004: Another controlled feeding will be conducted in postmenopausal women to test the interaction between meat protein and dietary calcium.

FY 2005: Blood and urine samples from above-mentioned study will be analyzed and the statistical analysis will be completed.

FY 2006: The results of the study will be reported in scientific meetings and in peer-reviewed journals.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
Technology has been transferred through a variety of activities by writing articles for the local newspaper, by speaking to various consumer and stakeholder groups and providing consultation to various media.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
Z.K. Roughead presented a paper entitled: "Effects of daily substitution of soy protein for meat protein on calcium retention and biomarkers of bone and cardiovascular health in postmenopausal women", at the 5th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease. Orlando, Florida. September, 2003. Information about the effects of soy protein on calcium retention was presented to postmenopausal women at University of North Dakota Women's Center and to members of North Dakota Stockmen Association.

Publications: **Log 115:**

1. Roughead, Z.K., Lykken, G.I., Johnson, L.K. Substituting soy protein for meat protein did not affect calcium (Ca) retention or biomarkers of bone and cardiovascular health in a controlled feeding study of healthy postmenopausal women. FASEB Journal. 2003. v.17 A705. Presented by Z.K. Roughead at Experimental Biology meeting. San Diego, CA. April, 2003. 0000141857

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: OPTIMAL MINERAL NUTRITION FOR PHYSIOLOGICAL AND PSYCHOLOGICAL DEVELOPMENT, FUNCTION AND HEALTH

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

Suboptimal mineral intakes can impair physiological and psychological development and function, broadly affecting health, fitness, work productivity, school performance, social and emotional adjustment, and quality of life. For example, iron and zinc restriction adversely affect energy metabolism during physical activity, while iron and zinc supplementation of at-risk populations improve cognitive performance. For many mineral elements, however, the potential benefits and mechanisms of action for physiological and psychological function have not been evaluated, and the effects of mediating factors such as age, sex, body composition, special diets, lifestyle and genetic factors are poorly understood. Of particular concern is the increasing incidence of obesity and other eating disorders, and the potentially adverse effects of restrictive diets adopted by millions of Americans. Obese individuals often have altered mineral status, including lower copper, iron and zinc in blood, while weight loss adversely affects mineral nutrition, particularly copper. A clear understanding of the contribution of mineral elements to optimal physiological and psychological development and function, and the influence of mediating factors, is necessary for establishing dietary recommendations applicable to all segments of the population, and for evaluating special diets, the efficacy of dietary supplements, and the potential benefits of value-added foods.

The objective of this research is to improve health and enhance quality of life by determining for healthy and at-risk (e.g., school-aged children, rural elderly, American Indians) populations nutrient intakes that optimize physiological and psychological development, function and health; to develop new functional bases for establishing mineral element requirements; to identify mechanisms of action; and, to determine the influence of sex, age, genetic, lifestyle and environmental factors on mineral element requirements.

Dietary intakes and biochemical indices of mineral status are related to physiologic (e.g., body composition, weight maintenance, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine importance of specific minerals for optimal function and development. A mobile field laboratory, community studies facilities, and a residential metabolic unit is used to conduct epidemiologic, supplementation, fortification, and controlled feeding studies with healthy and at-risk groups (e.g., school-aged children, rural elderly, American Indians). Randomized controlled trials evaluate the effects of graded intakes of minerals, such as zinc, copper, magnesium and boron, in the context of mediating factors (e.g., genotype, controlled stressors). Animal and cell culture studies enhance the efficacy of human studies and help determine the mechanisms of action of functional outcomes. New methods and technologies are developed to increase efficacy of physiological and psychological assessments and promote their use by other nutrition scientists. Studies of healthy adults and targeted

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populations (e.g., adolescents, rural elderly, American Indians) are complemented by animal studies. Studies involve university, industry and government collaborations.

2. **How serious is the problem? Why does it matter?**

Findings obtained during the last half century indicate that the mineral elements calcium, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal physiological and psychological function of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on function or to adequately characterize the relationship between mineral element nutrition and function. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. The consequences of mild to moderate mineral deficiencies for physiological and psychological development, function and health are also largely unknown. This represents a serious problem because suboptimal mineral intakes and status have been linked to chronic disorders such as cardiovascular disease, diabetes, obesity, depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals (e.g., calcium, copper, iron, magnesium, zinc) are less than recommended in many segments of the U.S. population and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly, American Indians). Existing data are typically inadequate or unavailable to make recommendations based on functional outcomes.

To respond to intense public interest in the relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a need to increase our knowledge of the functional consequences of varying intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Further, increased knowledge of the relationship between mineral element nutrition, physiological and psychological function is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and/or adverse effects associated with taking dietary supplements, a multi-billion dollar industry in the United States.

This project addresses the need for increased experimentally-derived knowledge leading to a better understanding of the relationships among mineral nutrition, physiological and psychological function. Such knowledge is critical to making recommendations for mineral intakes that will facilitate optimal development, health and performance throughout the life span in all segments of our population. Further, physiological and psychological performance are unique as a criteria for establishing nutritional adequacy because they represent the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess.

This research will provide needed information to establish recommendations for dietary intakes of mineral elements throughout the life span and to help American consumers choose foods that optimize physical and mental performance, social and emotional adjustment, and prevent or ameliorate chronic metabolic diseases such as obesity and Type 2 diabetes. Resulting information will also be useful to improve food assistance programs and to evaluate special diets, the efficacy of dietary supplements, and the potential benefits of value-added foods to maintain health and well-being. The products of this research will be new knowledge disseminated through scientific publications, books and monographs, presentations at scientific meetings, and articles in the lay media.

Primary customers for the products of this research are agricultural and commodity groups, the food industry, supplement manufacturers, policy makers, health and

nutrition professionals and the general public.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**

This research is directly related to the major components of the Human Nutrition Action Plan 107, "Nutrient Requirements", "Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease", and "Health Promoting Intervention Strategies for Targeted Populations". The research will address several priority objectives, including: (1) developing functional markers of mineral intakes and status; (2) identifying mechanisms of action for mineral elements; (3) determining the influence of genetic, environmental and lifestyle factors on mineral requirements; (4) evaluating the interaction of dietary, genetic, environmental and lifestyle factors on obesity and prevention of chronic disease; (5) identifying dietary intervention strategies effective with minority populations; and, (6) characterizing the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health. It is noteworthy that President Bush's "Healthier U.S." initiative (launched June 20, 2002) encourages healthy eating to help Americans to live longer and better lives. Also, a bill entitled "Improved Nutrition and Physical Activity Act (IMPACT)" was introduced in the Senate (July 30, 2002) to focus on the public to choose healthful foods and to exercise more frequently to combat development of chronic diseases.

4. **What were the most significant accomplishments this past year?**

A. Single Most Significant Accomplishment during FY 2003:

During 2003, we completed a study initiated in 2002 to evaluate culturally appropriate research methods and characterize basic relationships among mental and physical health, nutrition, physical activity, and social factors in Northern Plains Indians living on North Dakota's four reservations. Data collection was accomplished with a mobile research laboratory that traveled to eight powwows and health fairs held on the reservations. Preliminary analyses indicate that depression in NPI is strongly related to food insecurity and moderately related to less exercise, higher body mass index, and poorer physical health and diet, all moderated by gender. Impact: Findings will serve as a foundation for future studies to determine specific nutrient needs and the impact of diet and activity on health issues endemic to American Indian communities, with the goal of implementing effective, culturally appropriate, community-based interventions to improve health and quality of life; this study has also help build relationships between regional American Indian communities and the USDA, critical to the success of future nutrition studies.

B. Other Significant Accomplishments:

None.

C. Significant Accomplishments/Activities that Support Specific Target Populations:

See 4.A. above.

D. Progress Report:

Work began with the third cohort (fall of 2003) of subjects for a field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children, aged 12-14 years, attending local middle schools. During the fall of 2001 and 2002, 168 children drank fruit juice containing 0,10 or 20 mg zinc each school day for 10-12 weeks. Before and after the intervention, dietary intake, health status, cognitive function, psychoeducational performance, body composition and physical fitness are being evaluated; a mobile research laboratory is being used to help standardize

assessments and minimize disruption to school programs. Another 132 children will participate in the study during the fall of 2003 to complete the study. Findings will help determine needed zinc intakes to promote optimal physical and behavioral development of peri-pubescent adolescents at-risk for zinc deficiency because of rapid growth.

Currently underway are analyses and reporting of data from initial survey of Northern Plains Indians to identify relationships among nutrition, mental and physical health, physical activity, and social factors (see 4.A. above). Findings will serve as a foundation for future studies to determine specific nutrient needs and the impact of diet and activity on health issues endemic to American Indiain communities, with the goal of implementing effective, culturally appropriate community-based interventions to improve health and quality of life.

Previous studies in this and other laboratories have shown that mineral intakes affect menstrual symptomatology. We completed data collection and began data analysis in a study to determine whether calcium, magnesium and other mineral intakes and status mediate the stress responses (autonomic nervous system, corticosteroid concentration, prostaglandin production) of women with dysmenorrhea. Findings will help determine whether dysmenorrhea can be characterized by abnormal stress responses and whether these responses are affected by mineral nutrition.

Data collection continued in a multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in an ongoing controlled metabolic unit study. Findings will help determine needed intakes of copper and zinc for optimal cognitive and brain function, and healthy cardiovascular function in older individuals, and will clarify previously observed interactive effects of copper and zinc on memory function of older women.

Zinc is a limiting nutrient in the diets of adolescents, many of whom engage in physical activities to promote physical performance. A study was completed to determine the effects of dietary zinc restriction on activity and expression of carbonic anhydrase, a zinc-containing enzyme, in skeletal muscle in response to endurance exercise training. At a dietary level of zinc (4 mg/kg diet) consistent with that consumed by physically active individuals engaging in chronic endurance activities, carbonic anhydrase activity and protein concentrations in slow-twitch muscle were significantly decreased compared to rats fed a zinc-adequate (15 mg/kg) diet. This study also confirmed previous findings from our laboratory in which carbonic anhydrase isozyme activities in red blood cells were significantly decreased when dietary zinc was limiting (4.5 mg/kg). These findings show that dietary zinc adversely affects zinc-containing enzymes used to promote energy utilization and gas exchange between cells and the blood.

Reduced zinc status has been postulated to impair muscle function. A pilot study was conducted to evaluate the effects of moderate zinc deficiency on in situ muscle function. Rats fed a diet containing 4.5 compared to 15 mg zinc per kg of diet had reduced force production and endurance during electrical stimulation of the isolated hind-limb muscles. These observations suggest a key role of zinc in regulating muscle function in vivo. Additional work is needed to improve technical aspects of the experimental procedure before final confirmation of the role of zinc in muscle function can be stated.

Iron deficiency anemia has been shown in humans to result in impaired thermoregulatory capacity during acute cold exposure. To delineate the mechanisms of iron deficiency on regulation of thermogenesis, rats were fed graded dietary iron (5, 15 and 45 mg iron per kg diet) then acute exposed to cold air (3 degrees Centigrade for 6 hr). Anemic rats were hypothermic (decreased body temperature) with reduced voluntary activity compared to iron-adequate or iron-supplemented animals. Uncoupling proteins in the brown adipose tissue were significantly decreased in low-

iron rats in association with decreased catecholamines and thyroid hormone. Importantly, there was a linear relationship between iron status (hemoglobin) and uncoupling protein in brown adipose tissue. Although we hypothesized that anemia would elicit adverse brown adipose tissue metabolism, the relationship between hemoglobin and uncoupling protein expression suggests that marginal iron deficiency may be impairing thermoregulation. Additional work is needed to discriminate effects of marginal compared to severe iron deficiency on thermoregulatory capacity (e.g., thyroid hormone and catecholamine metabolism; uncoupling protein and heat shock protein expression and induction).

Methodology was developed for isolating viable, respiring mitochondria from brain and muscles. Development of methodology needed for quantifying protein carbonyls and nitrotyrosine in mirochondria and other cellular components was initiated.

Both boron and long-chain polyunsaturated fatty acids generated from alpha-linolenic acid (omega-3 fatty acids) have beneficial effects for many of the same life processes including eye development or function, apparently through affecting cellular membranes. Rats that were fed boron-deficient or -adequate diets containing either canola oil (high in omega-3 fatty acids) or palm oil (low in omega-3 fatty acids) through gestation and lactation produced pups that were fed the same diet for three weeks post-weaning. Examination of the eyes of pups by electron microscopic procedures revealed that a diet high in omega-3 fatty acids and boron improved mitochondrial configuration, condition, and abundance of cristae. The findings suggest that an adequate boron intake is important for obtaining the beneficial effect of long-chain omega-3 fatty acids on eye development.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

The increased emphasis by national and international public health agencies has stimulated the development of devices for routine, personal assessment of body composition. We evaluated the validity of commercially-available bioelectrical impedance devices that use foot-to-foot or hand-to-hand electrode placements to measure body fatness and compared the results with dual x-ray absorptiometry in 62 women and 48 men, 20 to 72 years of age. Compared to the reference method, the regional impedance instruments significantly underestimated body fatness except the foot-to-foot placement in the men. Impact: These findings indicate that regional bioelectrical impedance devices that are marketed to the public are inaccurate because of an unequal distribution of muscle between the upper and lower body more so in females than males.

6. **What do you expect to accomplish, year by year, over the next 3 years?**

FY 2004 - Complete first field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children, aged 12-14 years, attending local middle schools (described in 4.D. above). Analyze results and report findings. Findings will help determine needed zinc intakes to promote optimal physical and behavioral development of adolescents at-risk for zinc deficiency because of rapid growth, and provide a foundation for studies with other nutrients and elementary and high-school children.

Previous research suggests that cognition, psychosocial function and physical fitness may be sensitive indicators of suboptimal iron and zinc nutrition in children and adolescents, and that underserved groups (e.g., American Indians) may be at particular risk for inadequate intakes of iron and zinc. Use of these indicators to establish mineral requirements will improve the precision of recommended intakes for this age group. However, there is a paucity of scientific data on the health and functional effects of graded intakes of iron and zinc in children and adolescents. We will plan and begin recruitment of children in grades 4, 7 and 10 to determine the benefit, if any, of fortifying foods with iron and zinc for health, growth, body composition, muscle strength and endurance, psychomotor and

cognitive function, school performance and social adaptation. Emphasis will be placed on including schools on regional American Indian reservations. Outcomes will be used to identify efficacious intervention programs to enhance physical, psychological and social development. Relative sensitivity of existing and new measures of physiological and psychological function to mild and moderate copper, magnesium and zinc deficiencies and to graded intakes of these nutrients will be determined.

Complete data collection, analyze results and report findings of multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in a controlled metabolic unit study (described in 4.D. above). Findings will help determine needed intakes of copper and zinc for optimal cognitive and brain function, and healthy cardiovascular function in older individuals, and to verify previously observed interactive effects where moderately high zinc intakes impaired some aspects of memory in individuals with low copper intakes.

It is likely that copper, magnesium and zinc nutrition in the elderly affects risks for age-related diseases, body composition, cognitive function, social and emotional adjustment, and activities of daily living. It is also likely that the mineral status of institutionalized elders is inferior to that of free-living relatives and that suboptimal mineral status among institutionalized elderly is associated with impaired health and function. We will plan and initiate a survey of institutionalized (assisted living facility) elderly (>70 years) and non-institutionalized spouses/siblings (controls) to determine relationships among mineral element status, life-style factors, health, body composition, cognitive function, social and emotional adjustment, and daily activities. Outcomes will be used to implement supplementation or food fortification intervention trials with anticipated emphasis on potential beneficial effects of improved copper, magnesium and zinc nutrition on age-related physical and mental problems, including losses in muscle mass and bone mineral density and dementia (e.g., impaired memory, confusion and depression) affecting activities of daily living. Relative sensitivity of existing and new measures of physiological and psychological function to mild and moderate copper, magnesium and zinc deficiencies of these nutrients will be determined.

Initiate focus groups with Native community leaders and members to plan a health and nutrition survey similar to the NHANES to be conducted with a mobile research laboratory on American Indian reservations in the upper Midwest. Relationships among biochemical and clinical measures of mineral status, health, genetic and lifestyle factors, body composition and psychosocial function will be used to plan mineral supplementation or food fortification intervention trials with outcomes including improved management of metabolic disease symptoms. Socioeconomic and lifestyle factors, depression and other relevant behavioral and mood symptoms also will be evaluated. Findings will help determine the health consequences of mineral intakes and traditional diets in American Indians and facilitate future large-scale intervention studies in this at-risk population.

Complete data collection and analyses in ongoing study to determine the relative benefits for brain function and cognition of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China. This research is scheduled as part of a recently funded, long-term project on selenium bioavailability and function.

Continuing the work described in 4.D. above, plan and initiate a study of the effects of graded dietary zinc on skeletal muscle function. This study will improve procedures to use in situ muscle function testing to test the hypothesis that restricted zinc intake impairs muscle contraction and relaxation, strength and endurance and that the mechanism of action is calcium transport. Results will

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confirm the role of zinc in muscle function and serve to plan future human studies.

Continuing the work described in 4.D. above, plan and initiate a study in rats to determine the role of iron intake/status in the regulation of energy production by examining uncoupling protein and heat shock protein metabolism. Rats will be fed graded dietary iron and fat and exposed to thermoneutral conditions or an acute cold exposure. This work will improve ability to discriminate effects of marginal compared to severe iron deficiency on thermoregulatory capacity (e.g., thyroid hormone and catecholamine metabolism; uncoupling protein and heat shock protein expression and induction).

Design and implement a study in rats to determine the effects of graded dietary copper on reactive oxygen and nitrogen species on angiogenesis and skeletal muscle apoptosis as beneficial adaptive responses to exercise. Results will provide needed information to plan a human study of copper-dependent effects on physiological adaptation to aerobic exercise.

Animal studies will be planned and implemented to investigate the effects of copper deficiency during pregnancy on the production of reactive oxygen species by brain and muscle mitochondria in neonates. It will be determined whether copper deficiency during pregnancy leads to increased generation of reactive oxygen species by brain and muscle mitochondria in the offspring the copper-deficient dams. If increased mitochondrial production of reactive oxygen species occurs, it will be determined whether the altered cellular redox state increases oxidative carbonylation and nitrosylation of mitochondrial and extra-mitochondrial proteins.

An experiment with animals will be performed that will determine whether a dietary deprivation of boron impairs sensory functions and brain development and whether impairments are exacerbated by an inadequate intake of long-chain omega-3 fatty acids.

FY 2005 - Implement study of 4th, 7th and 10th grade children initiated in FY2004 (see above) to determine the possible benefits of iron and zinc fortification for physical, psychological and social development. It is anticipated that data collection from all age groups will require a minimum of 3 years.

Implement study of institutionalized and non-institutionalized elderly initiated in FY2004 (see above) to determine the relationship between nutrition, health and function.

Conduct survey, analyze results and report findings of survey of American Indians planned in FY2004 to determine the health consequences of mineral intakes and traditional diets in American Indians and facilitate future large-scale intervention studies in this at-risk population.

Plan and initiate a community feeding study to determine relationships between zinc intake, strength gain and body composition change of adults during resistance training. In collaboration with the University of North Dakota, healthy adults will be randomized and fed graded dietary zinc and participate in controlled resistance training. Outcome measures will include strength gain, muscle mass accretion, zinc status, and anabolic hormone responses. Findings will contribute new information about adaptation of zinc metabolism and status in response to strength gain and muscle mass accretion.

Animal studies will be conducted to determine whether oxygen radicals generated within heart mitochondria during copper deficiency can cause progressive brain and muscle pathology through permanent mitochondrial dysfunction caused by the induction of mutations in mitochondrial DNA. It will be determined whether different types of dietary carbohydrates, lipids, and antioxidants can potentiate or ameliorate oxidative damage in the heart caused by copper deficiency.

FY 2006 - Continue to implement study of 4th, 7th and 10th grade children initiated in FY2004 (see above) to determine the possible benefits of iron and zinc fortification for physical, psychological and social development. It is anticipated that data collection from some age groups may be completed during this year.

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Analyze data and report findings from study of institutionalized and non-institutionalized elderly initiated in FY2004 (see above), and begin planning mineral supplementation or food fortification intervention trials with institutionalized and non-institutionalized elderly to experimentally determine the benefits of improved copper, magnesium and zinc nutrition on age-related physical and mental problems, including losses in muscle mass and bone mineral density and dementia (e.g., impaired memory, confusion and depression) affecting activities of daily living. The mediating effects of prior mineral element status, life-style factors, health, body composition, cognitive function, social and emotional adjustment, and daily activities also will be evaluated. Relative sensitivity of existing and new measures of physiological and psychological function to mild and moderate copper, magnesium and zinc deficiencies and to graded intakes of these nutrients will be determined.

Initiate focus groups with Native community leaders and members to review results from survey completed in FY2005 (see above) to plan effective, culturally appropriate, community-based dietary and physical activity interventions to improve health problems endemic to Native populations, including cardiovascular disease, diabetes, obesity and depression.

Male and female animals of different ages will be used to determine: 1) the amount of dietary copper required to increase aerobic capacity during endurance training; 2) the amount of dietary zinc required to increase muscle strength during resistance training; and, 3) whether stressors such as physical exercise exacerbate the negative effects of copper and zinc deprivation by adversely affecting brain structure and function.

In order to correlate oxidative damage caused by copper deficiency with biochemical perturbations in brain and muscle function, oxidatively modified proteins will be identified and associated changes in the biological functions of the modified proteins will be characterized. This will include alterations in signaling pathways caused by nitrosylation of protein targets phosphorylated by tyrosine kinases.

An experiment will be performed with experimental animals to determine the effect of dietary nickel on sensory and psychomotor functions is influenced by the dietary fatty acid composition of the diet.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

Science and technology transfer through presentations at professional meetings, including:

Gray, J.S., Penland, J.G., Wilson, E., Lambert, P. Depression and Moderating Factors in Northern Plains Indians. Presented by J. Gonzalez at the Annual Meeting of the American Psychological Association, Toronto, Canada. August 2003.

Gray, J.S., Penland, J.G., Wilson, E., Lambert, P. Nutrition, food security, depression, and quality of life in Northern Plains Indians. Presented by J.S. Gray at the Society of Indian Psychologists meeting, Logan, UT. June 2003.

Gray, J.S., Penland, J.G. Presented by J.S. Gray at the Joint Conference of the Higher Education Resources Organization for Students (HEROS) and North Dakota Indian Education Association (NDIEA), Bismarck, ND. September 2003.

Lukaski, H.C. Who are you calling fat? Lecture presented at the Department of Nutrition and Dietetics - Clinical Practicum, University of North Dakota, Grand Forks, ND. September 2002.

Lukaski, H.C. Validation of fan-beam DXA - comparisons with chemical analyses in animal models. Lecture presented at the Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health QDR

4500A Workshop, Rockville, MD. September 2002.

Lukaski, H.C. Zinc and muscle function - implications for rehabilitation and space travel. Lecture presented at the Human Physiology Section, Scientific Institute of Santa Lucia, University of Rome Tor Vergata, Rome, Italy. October 2002.

Lukaski, H.C. Regional bioelectrical impedance analysis - applications in health and medicine. Lecture presented at the Sixth International Symposium on In Vivo Body Composition Studies, Villa Mondragone, Rome, Italy. October 2002.

Lukaski, H.C. Role of minerals in preservation of bone during weight loss in the obese. Lecture presented at the Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health, Addressing the Obesity Epidemic Conference, Denver, CO. November 2002.

Lukaski, H.C. Adverse effects of low dietary magnesium and zinc on cardiorespiratory function during exercise. Lecture presented at the Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO, January 2003.

Lukaski, H.C. Bioelectrical impedance analysis - clinical applications and challenges. Lecture presented at the Clinical Nutrition Research Center, Vanderbilt University Medical Center, Nashville, TN. June 2003.

Moulton, P.L., Penland, J.G., Petros, T.V., Carr, P.A., Dahlen, G.M., King, B.M., Apostal, K.J., Zaruba, R.A., Saari, J.T. Impact of chronic corticosterone and zinc deprivation on behavior and hippocampal degeneration. Presented by P.L. Moulton at the Society for Neuroscience meeting. Orlando, FL. November 2002.

Moulton, P.L., Petros, T.V., Apostal, K.J., Park II, R.V., Ronning, E.A., King, B.M., Penland, J.G. Ethanol-induced memory impairment and post-intoxication facilitation of memory. Presented by P.L. Moulton at the Annual Meeting of the Midwestern Psychological Association, Chicago, Illinois. May 2003.

Nielsen, F.H. Does boron have an essential function similar to an omega-3 fatty acid function? Invited plenary talk at the 21st Workshop on Macro and Trace Elements, Jena, Germany. October 2002.

Penland, J.G. Iron, zinc and other essential minerals affect cognitive function and behavior throughout the life span. Invited address at the American Dietetics Association¿s Everyday Solutions Retreat 2002. White Sulphur Springs, WV. November 2002. Attendees were ADA¿s media spokespersons.

Poltavski, P., Petros, T., Penland, J.G. Effects of transdermal nicotine on prose memory and attention. Presented by P. Poltavski at the Annual Meeting of the Midwestern Psychological Association, Chicago, Illinois. May 2003.

To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been adapted for use with children, adolescents, elderly, American Indians, non-English speaking persons (Spanish, Chinese, French), and other groups at risk for mineral deficiency (e.g., athletes). This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign. Currently in progress are 3 collaborative research projects using this technology, supported by 2 granting agencies, and involving principal investigators in China and the U.S.

Identification and refinement of reliable and valid measures of suboptimal mineral status and function represent ongoing challenges to the success of this research, as well as one of its most important potential benefits. Lack of familiarity with

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physiological and psychological assessments, the need for careful training of test administrators, and the lack of age-, sex- and ethnic-specific norms are additional constraints on the success of this research.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**

Presentations to Nonscientific Groups:

Penland, J.G. Zinc fortification effects on cognition, psychoeducational performance, body composition, physical fitness and immune function of adolescents. Presented to the Grand Forks Public School Board. Grand Forks, ND. May 2003.

Media Coverage:

Bliss, R.M. Driving nutrition research to the community. Agricultural Research Magazine. 2003. v. 51(1). p. 11-13. (news magazine article). Article featured research conducted by J.G. Penland and H.C. Lukaski.

Penland, J.G. RDA and DRI: they're not just acronyms! Grand Forks Herald. June 4, 2003, Section D, p. 1. (newspaper column)

Quill, S. Alternative energy: can supplements supply what your diet won't? Men's Health. July-August 2003. p. 126.

Wood, M. On-the-go nutrition van reaches more volunteers. Food & Nutrition Research Briefs. April 2003. p. 3. (government newsletter) Article featured research conducted by J.G. Penland and H.C. Lukaski.

This research program, including study results, frequently receives public exposure through coverage by the popular press (local, regional and national newspapers, magazines, radio and television) and industry newsletters and magazines. For example, H.C. Lukaski was cited in news stories published by United Press International and on ESPN News on the mutagenic effects of chromium picolinate on body composition, and J.G. Penland was cited in stories appearing in First for Women, Natural Health and Redbook magazines on the effects of copper on sleep, magnesium on brain function, and selenium on mood.

Publications: **Log 115:**

1. Klevay, L.M., Lukaski, H.C., Britton, S.L., Kock, L.G. Increased copper and iron in skeletal muscles of rats bred to run long distances. FASEB Journal. 2003. v. 17. Abstract. p. A1160. Presented by L.M. Klevay at the Experimental Biology Meeting. San Diego, CA. April 2003. 0000141855
2. Lukaski, H.C., Hall, C.B., Michelsen, K.G. Effects of dietary zinc and exercise training on zinc status and carbonic anhydrase activities. FASEB Journal. 2003. v. 17. Abstract. p. A695. Presented by H.C. Lukaski at the Experimental Biology Meeting. San Diego, CA. April 2003. 0000141320
3. Chumlea, W.C., Guo, S.S., Kuczmarski, R.J., Flegel, K., Johnson, C.L., Heymsfield S., Lukaski, H.C., Friedl, K., Hubbard, V.S. Body composition estimates from NHAMES III bioelectrical impedance data. International Journal of Obesity. 2002. 26:1596-1609. 0000124730
4. Sun, S.S., Chumlea, W.C., Heymsfield, S.B., Lukaski, H.C., Schoeller, D.A., Friedl, K., Kuczmarski, R.J., Flegel, K.M., Johnson, C.L., Hubbard, V.S. Development of bioelectrical impedance prediction equations for body composition with the use of a multi-component model for use in epidemiologic surveys. American Journal of Clinical Nutrition. 2003.v.77.p.331-340. 0000124731
5. Nielsen, F.H. Does boron have an essential function similar to an omega-3 fatty acid fucntion? Anke, M., Muller, R., Schafer, U., Stoeppler, M., editors. Schubert-Verlag, Leipzig, Germany. Macro and Trace Elements (Mengen- 0000139342

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und Spurenelemente). 2002.p.1238-1250.

6. Penland, J.G., Lukaski, H.C., Gray, J.S. Zinc fortification and cognitive and psychosocial function in young adolescents. FASEB Journal. 2003. v. 17. Abstract. p. A1087. Presented by J.G. Penland at the Experimental Biology Meeting. San Diego, CA. April 2003. **0000141325**

7. Yokoi, K., Egger, N.G., Ramanujam, V.M.S., Alcock, N.W., Dayal, H.H., Penland, J.G., Sandstead, H.H. Associations between iron and zinc nutriture in premenopausal women. FASEB Journal. 2003. v. 17. Abstract. p. A302. Presented by K. Yokoi at the Experimental Biology Meeting. San Diego, CA. April 2003. **0000141979**

8. Siders, W.A., Lukaski, H.C. Body, half-body and preventing limb bone-free, fat-free weight: Determinates of variability in bioelectrical impedance. Proceedings of the North Dakota Academy of Science. 2003. v. 57. p. 34. **0000148501**

Approved: ROOS ERIC E **Date:** 09/26/2003

Project Number: 5450-51530-008-01T **Accession:** 0406520 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: DETERMINATION OF THE EFFECTS OF BUCKWHEAT ON MANAGEMENT OF PRE-DIABETES AND NON-INSULIN DEPENDENT DIABETES (NIDDM) IN ANIMAL MODELS

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
Diabetes and obesity are public health problems that are growing at alarming rates in the United States. It is estimated that 17 million Americans, 6.2% of the population, have diabetes. Whereas 11 million individuals are diagnosed with this disease, another 6 million are unaware that they have the disease. Furthermore, an additional 16 million Americans have pre-diabetes, a condition that will eventually lead to diabetes. The majority of individual with diabetes have type 2 diabetes which is characterized by insulin resistance (a condition in which the body fails to properly use insulin), combined with relative insulin deficiency. Approximately 90-95% (16 million) of Americans who are diagnosed with diabetes have type 2 diabetes.

Certain foods have chemical components that may improve glucose utilization and insulin action. Buckwheat is a food that contains fagopyritols that are degraded to yield D-chiro-inositol which has been shown to act as a mediator of insulin action.

The objective of this research is to determine if fagopyritols in buckwheat can prevent the development of glucose intolerance and insulin resistance in rodent models of obesity and type 2 diabetes.

2. **How serious is the problem? Why does it matter?**
Although international and American public health organizations and agencies emphasize the need to improve diet as one approach to reduce chronic health problems including obesity, diabetes and cardiovascular disease, there is limited information about the health-promoting characteristics of many common foods. Buckwheat is potentially important commodity because it contains fiber known to reduce lipids and glucose and fagopyritols which are precursors of inositol phosphates. D-chiro-inositol is an inositol phosphate which when supplemented to the diet has been shown in animal studies to attenuate insulin resistance in diabetic animals. In one human study, large doses of supplemental D-chiro-inositol improved glucose utilization during an oral glucose tolerance test and enhanced ovulation in women with polycystic ovary disease. Thus, there is a need to determine the health-promoting value of buckwheat in prevention of and intervention with disorders of glucose and insulin metabolism, as well as to determine mechanisms of action.

Primary customers for the products of this research are agricultural and commodity groups, food technologists, health and nutrition professionals and the general public.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
Program 107, Human Nutrition (100%). This research is directly related to the major

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components of the Human Nutrition Action Plan, "Diet, Genetics, Lifestyle and the Prevention of Obesity and Disease".

4. **What were the most significant accomplishments this past year?**
D. Progress Report:

This report serves to document research conducted under a Trust Fund Cooperative Agreement, #58-5450-3-402, between ARS and MinnDak Growers. Additional details of research can be found in the report for the parent project 5450-51530-008-00D.

A preliminary study was conducted to evaluate the effects of uncooked compared to cooked farinetta (buckwheat flour) on the growth of young rats. After 30 days, rats fed uncooked farinetta experienced a reduction in growth rate compared to other rats fed the cooked farinetta or a control diet. The reduced growth persisted for the next 14 days. These findings resolved the question of the form of farinetta (cooked versus uncooked) needed for future studies of the effect of farinetta on indices of glucose and insulin metabolism.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
See 4D above.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
FY 2004 - Studies will be conducted in animal models of obesity/diabetes to determine the dose of farinetta, and thus fagopyritols, to improve glucose and insulin responses to controlled administrations of glucose (oral glucose tolerance testss and meal-feeding glucose challenges). Mechanisms of action will be evaluated including glucose transporters (GLUT2 and GLUT4) in perifpheral tissues as well as intracellular insulin signaling mechanisms.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
None.

Publications: Log 115:

Approved: ROOS ERIC E **Date:** 09/22/2003

MINERAL NUTRIENT INTAKE AND MECHANISMS OF UTILIZATION FOR HEALTH MANAGEMENT UNIT

Project Number: 5450-51000-031-00D **Accession:** 0404974 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: ROLE OF DIETARY MINERALS ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
Improving the diet by increasing the consumption of whole grains, fruits and vegetables may decrease the incidence of cancer by 30-40%. Although fiber, vitamins and phytochemicals have received the most attention as chemopreventive components of a diet rich in grains, fruit and vegetables, minerals also may be important chemopreventive components. For example, human epidemiologic and supplementation studies, as well as extensive animal studies, have shown the efficacy of selenium in cancer prevention. Food contains different chemical forms of selenium as well as other dietary constituents which will influence the chemopreventive effect of selenium. Furthermore, recent studies suggest that dietary copper protects against colon cancer in several animal models. Other dietary minerals may be beneficial but their role in cancer prevention has not been thoroughly investigated. Mammary, colon and prostate cancers are the main types of cancer which are influenced by dietary factors. A key to understanding the relationship between optimal mineral intake and cancer is determining the effects of mineral intake on cellular processes such as gene expression, oxidative stress, apoptosis and signal transduction. Studies are and will be conducted to determine whether mineral elements such as selenium, copper, and zinc affect biomarkers of carcinogenesis, including carcinogen-induced aberrant crypt formation (a preneoplastic lesion for colon cancer), carcinogen-DNA adduct formation, oxidative status, selenoprotein and detoxifying enzyme activities, DNA methylation and tumor development. Min (multiple intestinal neoplasia) mice will be used to study the effects of trace minerals on the pathogenesis of intestinal cancer in a genetic model for cancer susceptibility. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower but there is gene specific hypermethylation and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Global DNA methylation, gene specific DNA methylation, methyl metabolism and DNA methyltransfease activity will be evaluated in colon-derived human cells cultured in medium containing different chemical forms of selenium and different concentrations of folate, iron, or zinc and in animals fed diets containing different amounts of selenium, folate, iron or zinc. To determine the mechanisms for the chemopreventive effects of selenium and copper against colon cancer, gene specific macroarrays will be utilized and the effects of copper and selenium on signal transduction pathways for apoptosis and regulation of the cell cycle will be examined in cultured cells. The biological activity of zinc transcription factors will be studied using electrophoretic mobility shift assays or reporter gene constructs. Controlled human feeding studies and/or supplementation studies have and will be conducted to determine whether trace minerals shown to affect carcinogenesis in animal and cell culture models affect cancer susceptibility in humans. Humans will be fed different diets and fecal water will be analyzed for cytotoxicity, apoptosis, genotoxicity, free radical production and alkaline

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phosphatase activity. Lymphocytes will be analyzed for DNA methylation, expression of cancer related proteins and measures of oxidative stress/status. Serum from animals or humans fed different concentrations of trace minerals will be used in cell culture systems to investigate cancer susceptibility.

2. **How serious is the problem? Why does it matter?**
Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease is approaching $200 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 30-40% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake is one factor that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
This research is related to National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research will help identify biomarkers relating the development of cancer to trace element intake by elucidating mechanisms through which trace elements impair cellular function. This is relevant to Component 1: Nutrient Requirements because one of the priority objectives is to adapt current methods or develop new methods to identify specific disease preventing bioactive dietary factors and elucidate their mechanisms of action. Another priority objective is to use the biomarkers as screening tools to identify the specific bioactive factor(s) responsible for the effects. This research is also relevant to Component 2: Diet, Genetics, Lifestyle and the Prevention of Disease. This research will identify the nutrient-relevant influences on gene expression that have consequences on human health and disease.

4. **What were the most significant accomplishments this past year?**
A: Single Most Significant Accomplishment during FY 2003 year:

The mechanisms by which the aqueous phase of human feces (fecal water) can positively or negatively affect cell cycle progression in colon cells during tumor development are not completely understood. We studied possible mechanisms with the goal of developing sensitive biological indicators for colon cancer risk by using easily obtainable fecal water. Our results indicate that human fecal water, which can be altered by dietary components, can induce genes that affect cell division and may be important in protection against colon cancer. One of these genes appears to be uniquely and highly sensitive to human fecal water; thus, it is being further studied as a possibly marker of colon cancer.

B. Other Significant Accomplishment(s), if any:

Selenium is a component of several antioxidant enzymes and regulation of some of these enzymes may be linked to the ability of selenium to protect against cancer. Reporter gene constructs of thioredoxin reductase, a seleno-enzyme, were produced at the GFHNRC and inserted into human liver cells that were then incubated with multiple compounds associated with protection against oxidative stress. Reporter genes were activated in a manner that suggested the presence of a regulatory gene sequence called the Antioxidant Responsive Element (ARE); mutation and gel shift analysis confirmed the presence of this sequence. This study demonstrates the

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seleno-enzyme thioredoxin reductase is part of a large family of genes that are coordinately regulated in times of oxidative stress by the Antioxidant Responsive Element, and thus thioredoxin reductase may help decrease oxidative damage-induced carcinogenesis.

Many observations suggest an important role for DNA methylation in cancer pathogenesis. We demonstrated that dietary selenium and folate interact to affect DNA methylation and one-carbon metabolism. Our studies showed that selenium and folate interacted to influence one-carbon metabolism and cancer susceptibility such that the number of aberrant crypts and the concentrations of plasma homocysteine and liver S-adenosylhomocysteine were the highest and the concentrations of plasma folate and liver S-adenosylmethionine and the activity of liver methionine synthase were the lowest when rats were fed deficient folate and supplemental selenium. These results suggest that understanding this interaction between selenium and folate may help determine the mechanism of cancer protection afforded by folate and high dietary or supplemental selenium and, that when studying folate, selenium status should be monitored and, vice versa, when studying selenium, folate status should be monitored.

C. None.
D. None.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

Inadequate dietary copper has been shown to increase the frequency of carcinogen-induced preneoplastic (precancerous) lesions (aberrant crypt foci) associated with colon cancer development in rats and the amount of intestinal tumors in a mouse model for human colon cancer susceptibility suggesting that low dietary copper may be a possible dietary factor that increases the susceptibility to colon cancer in humans. A controlled feeding study was conducted in order to investigate the effects of low and adequate copper intakes on copper nutriture and putative risk factors for colon cancer in healthy men. Low dietary copper significantly increased fecal free radical production and alkaline phosphatase activity but did not affect fecal water volume, pH or hematological indicators of copper status. These results suggest that low dietary copper adversely affects fecal free radical production and fecal water cytotoxicity and alkaline phosphatase activity which are putative risk factors for colon cancer.

Demonstrated that selenium is effective in the prevention of chemical carcinogen-induced aberrant colonic crypt foci, preneoplastic lesions indicative of colon cancer. Aberrant crypt foci development was inhibited in a dose dependent fashion that was also dependent on the form of selenium. The same pattern of inhibition of aberrant crypts by different chemical forms of selenium was also noted for the inhibition of carcinogen-induced DNA-adducts, suggesting that the inhibition of DNA-adduct formation may be a mechanism for the overall chemopreventive effect of selenium. Demonstrated that selenium from high-selenium broccoli was more effective than salt or amino acid forms of selenium for prevention of colon cancer. Found that high-selenium broccoli sprouts were as efficacious as broccoli florets for prevention of colon cancer.

Familial adenomatous polyposis (FAP) is a disease that has been linked to changes in the APC gene known as mutations; individuals possessing these mutations develop numerous intestinal polyps (precancerous lesions) at an early age. Min mice (multiple intestinal neoplasia mice) carry a mutation in what is equivalent to the human APC gene and develop intestinal tumors similar to those found in patients with familial adenomatous polyposis syndrome and are therefore a good model for the investigation of the effects of dietary alterations on genetic susceptibility for intestinal cancer. Min mice that were fed selenium-enriched broccoli had a

significantly lower small intestine tumor incidence and a significantly lower small intestine tumor burden than animals fed adequate dietary selenium as selenite. These findings suggest that inadequate selenium-enriched broccoli can decrease tumor development in a genetic model for human cancer.

Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Studies were conducted to determine whether a DNA methyltransferase inhibitor would alter the effect of dietary selenium on the formation of aberrant crypts, a preneoplastic lesion for colon cancer. Animals fed a selenium-deficient diet had a significantly higher number of aberrant crypts than animals fed adequate dietary selenium; however, when animals were injected with a DNA methyltransferase inhibitor, there was a significant reduction in aberrant crypt formation and dietary selenium did not affect aberrant crypt formation. These results suggest that decreased DNA methyltransferase activity may protect selenium deficient animals against colon cancer susceptibility.

The elucidation of the mechanisms by which selenium regulates the cell cycle can lead to a better understanding of the nature of selenium's essentiality and its role in disease prevention. The effects of selenium deficiency or adequacy on HL-60 (a human lymphocytic cell line) cell progression were examined in serum-free media. Cell cycle analysis revealed that selenium deficiency inhibited cell cycle progression; gene analysis revealed that selenium deficiency decreased the mRNA expression of many cell cycle regulatory genes; and phosphorylation analysis demonstrated that selenium deficiency decreased the phosphorylation state of total cellular protein. Collectively, these results suggest that selenium is critical for human lymphocyte cell division, growth and prevention of cell death.

The frequency of carcinogen induced aberrant crypt foci was significantly increased in animals fed low dietary copper and tended to be increased in animals fed low dietary manganese and high dietary iron. Altered activities of antioxidant enzymes, known as superoxide dismutases, were significantly correlated with the number of the anatomical lesions associated with colon cancer. These findings suggest that dietary alterations which affect superoxide dismutase activity affect cancer susceptibility.

Demonstrated that low dietary copper increased the formation of preneoplastic lesions for colon cancer and decreased protein kinase C expression, a series of proteins involved in the signal transduction pathway within the cell. These results provide a biochemical explanation for the increased incidence of precancerous lesions in colons of copper-deficient rats when they are challenged with a carcinogen.

6. **What do you expect to accomplish, year by year, over the next 3 years?**

In FY 2004, sample collection of the two human feeding studies will be completed (a controlled human feeding study investigating the effect of wheat cereals containing various concentrations of selenium on selenium status, antioxidant status, cytotoxocity of fecal water and measures of colonic fermentation; a controlled feeding study investigating the effects of consumption of broccoli high in sulforpahane and selenium on oxidative status and biomarkers of cancer susceptibility). Animal studies will be conducted investigating the effects of different food versus chemical forms of selenium on carcinogen-DNA adduct formation in the colon and prostate. Animal studies will be conducted to determine the effect of dietary selenium and zinc on SelR and Msra. These findings will be correlated with markers of oxidative stress and biomarkers of cancer susceptibility. A search for genes with altered methylation patterns as a consequence of selenium status (or form of dietary selenium) will be started. Genomic and proteomic approaches will be

utilized to further define the effect of human fecal water and other trace elements on cell cycle and apoptosis in cultured cells, and to determine the impact of mineral status on the biological activity of zinc finger transcriptional factors, which could lead to the identification of molecular bio-marker gene candidates. Buckwheat is a cereal crop that contains rutin and quercitin, both powerful antioxidants, and preliminary studies have shown that buckwheat reduces the incidence of some cancers. We will study the anti-carcinogenic benefit of buckwheat in the aberrant crypt model of colon cancer.

In FY 2005, analysis of the human feeding studies conducted in FY 2003 and 2004 will be completed. Animal studies will be conducted to identify and further characterize genes that have altered methylation patterns as a result of selenium status (and through the interaction of dietary selenium and folate). Similar studies will be undertaken to determine DNA methylation differences in the long living Ames dwarf mouse. These experiments will include the use of restriction landmark genome scanning (RLGS) to determine the differences in DNA methylation as a result of dietary selenium or mouse type. Further animal studies will be conducted to determine the effect of form of selenium and concentration of dietary selenium on amount (message and protein) and activity of SelR. Studies using siRNA knockdown of various selenoproteins will be completed. These studies will include the use of single and multiple knockdowns in cell culture and will be used to determine the importance of these various selenoproteins in carcinogenesis. Cell culture studies will be initiated to determine how quinone oxidoreductase and thioredoxin reductase, two enzymes regulated in part through the antioxidant response element (ARE), are coordinated in response to oxidative stress.

In Fiscal year 2006, animal studies will be conducted to determine the effect of cross-breeding mice with a knocked out selenoprotein (most likely GPx) to mice that are prone to a specific cancer (mammary cancer, for example). These studies will definitively ascertain the importance of the selenoprotein for a particular cancer. Complete RLGS studies in the selenium and dwarf mice experiments started in FY 2005.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
   Information about the effect of trace minerals on cancer susceptibility as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national meetings and professional publications.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
   Uthus, E.O. Epigenetics, cancer, SAM and your diet. Grand Forks Herald, 1/1/03.

   Zeng, H. Fruits, vegetables pack cancer-fighting punch. Grand Forks Herald, 7/2/03.

Publications: **Log 115:**

1. Davis, C.D., Finley, J.W. Chemical versus food forms of selenium in cancer prevention. In: Functional foods and nutraceuticals in cancer prevention. Ed. Watson, R., Iowa State Press, Ames, Iowa. 2003, pp. 55-85. 0000137058
2. Uthus, E.O., Brown-Borg, H.M. Altered methionine metabolism in long living Ames dwarf mice. Experimental Gerontology. 2003. 38: 491-498. 0000140535

Project Number: 5450-51000-031-00D **Accession:** 0404974 **FY:** 2003

3. Uthus, E.O. Simultaneous detection of S-adenosylmethionine and S-adenosylhomocysteine in mouse and rat tissues by capillary electrophoresis. Electrophoresis. 2003. 24: 1221-1226. 0000138348

4. Davis, CD. Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men. Journal of Nutrition. 2003. 133:522-527. 0000137054

5. Zeng, H., Davis, C.D., Finley, J.W. Effect of selenium-enriched broccoli diet on differential gene expression in Min mouse liver. Journal of Nutritional Biochemistry. 2003. 14: 227-231. 0000136532

6. Zeng, H., Davis CD. Down-regulation of proliferating cell nuclear antigen gene expression occurs during cell cycle arrest induced by human fecal water in colonic HT-29 cells. Journal of Nutrition. 2003. 133: 2682-2687. 0000146994

7. Ren, J., Hintz, K.K., Roughead, Z.F., Duan, J., Colligan, P.B., Ren, B.H., Lee, K.J., Zeng, H. Impact of Estrogen Replacement on Ventricular Myocyte Contractile Function and Protein Kinase B/Akt Activation. American Journal of Physiology Heart and Circulation Physiology. 2003. 284: H1800-1807. 0000145609

8. Uthus, E.O., Davis, C.D. Dietary arsenite affects dimethylhydrazine (DMH)-induced aberrant crypt formation in colon and liver global DNA methylation of rats. FASEB Journal. 2003. v. 17: A1152. 0000141345

9. Davis, C.D., Uthus, E.O. Dietary selenium (Se) and folate affect dimethylhydrazine (DMH)-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. FASEB Journal. 2003. v. 17: A1371. 0000141851

10. Davis, C.D. Deficient dietary selenium (Se) adversely affects putative risk factors for colon cancer susceptibility in rats. FASEB Journal. 2003. v. 17: A1134. 0000141831

11. Zeng, H., Davis, C.D., Finley, J.W. Effect of selenium-enriched broccoli diet on differential gene expression in Min mouse liver. FASEB Journal. 2003. v. 17: A1159. 0000141638

12. Davis, C.D., Uthus, E.O. Selenium and homocysteine in humans. Journal of Nutrition. 2003. v. 133, p. 2392. 0000147612

Approved: ROOS ERIC E **Date:** 09/26/2003

Project Number: 5450-51000-031-01R **Accession:** 0406009 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: DETERMINATION OF NUTRIENT EFFECTS ON CANCER SUSCEPTIBILITY ON EPIGENETIC PROCESSES IN ANIMAL MODELS

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
Cancer is a manifestation of both abnormal genetic and epigenetic events. Epigenetic events represent a mechanism by which gene function is selectively activated or inactivated through non-mutagenic processes. A variety of regulatory proteins including DNA methyltransferases, methyl-CpG binding proteins, histone-modifying enzymes, chromatin remodeling factors, and their multi-molecular complexes are involved in the overall epigenetic process. Because epigenetic events are susceptible to change, they represent excellent targets to explain how environmental factors, including diet, may modify cancer risk and tumor behavior. Abnormal DNA methylation patterns are hallmarks of most cancers, including those of high prevalence in the United States i.e., colon, lung, prostate, and breast cancer. Recent evidence suggests that diet could be a key regulator of DNA methylation.

Selenium is a dietary trace element that is recognized as having potential anticancer properties. Interest in selenium as a prostate cancer preventative nutrient is showcased by the recent initiation of the SELECT trial by NCI. While selenium is known to modify several aspects of the cancer process, the mechanism(s) for bringing about a phenotypic change remains to be established. A possible mechanism of action of selenium is through its effect on DNA methylation.

Probing studies about the activity of selenium in regulating DNA methylation processes, as well as interactions between selenium and other nutrients known to influence methyl supply, i.e., folate, remain to be performed. The outcome of this collaboration will assist in the elucidation of mechanism(s) by which specific dietary factors influence DNA methylation processes as well as increasing our understanding of these processes in cancer prevention.

The objective of this research is to examine the interactive effects of dietary selenium and folate on cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using an animal system.

2. **How serious is the problem? Why does it matter?**
Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease is approaching $200 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 30-40% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients resulting in a healthier population and diminished health care

expenditures.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
This research is related to National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research will help identify biomarkers relating the development of cancer to trace element intake by elucidating mechanisms through which trace elements impair cellular function. This is relevant to Component 1: Nutrient Requirements because one of the priority objectives is to adapt current methods or develop new methods to identify specific disease preventing bioactive dietary factors and elucidate their mechanisms of action. Another priority objective is to use the biomarkers as screening tools to identify the specific bioactive factor(s) responsible for the effects. This research is also relevant to Component 2: Diet, Genetics, Lifestyle and the Prevention of Disease. This research will identify the nutrient-relevant influences on gene expression that have consequences on human health and disease.

4. **What were the most significant accomplishments this past year?**
A. None

B. None

C. None

D. A preliminary study was conducted to determine the feasibility of using a technique to scan thousands of genes for differences in DNA methylation (and thus cancer susceptibility) in rats fed graded amounts of dietary selenium and folate. This was the first time this technique (restriction landmark genome scanning) had been used to determine whether diet alone can affect methylation in promoter regions of DNA. The results indicate that dietary selenium and folate can affect specific DNA methylation patterns. Future studies will confirm and expand upon this and be used to identify the specific genes that are affected by dietary factors (e.g., selenium and folate) that have anticancer properties.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
See 4D.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
FY 2004 - Studies using restriction landmark genome scanning (RLGS) will be conduced in animal models to determine the effect of dietary selenium (form and does) and folate on gene promoter region DNA methylation. Similar studies will be initiated that use animal models of colon cancer. These studies will determine how diet affects the cancer process as measured by RLGS. Techniques to identify the affected genes will be initiated. Studies are planned to ascertain the mechanism of selenium's effect on methyl metabolism and how this affect is altered or enhanced by folate.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None.

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8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
None.

Publications: Log 115:

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-00D **Accession:** 0405026 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: MINERAL UTILIZATION & BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS & AGRICULTURAL PRACTICE

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
The nutritional and health status of the human population of the United States could be adversely affected by current and proposed changes in the composition of the diet. Such changes include 1) an emphasis on plant-based diets with limited intake of red meat, 2) nutrient fortification or supplementation of diets as proposed by health care professionals, or independently initiated by producers, and 3) production of genetically- or chemically-modified foods. This project will determine whether proposed changes to the US diet may affect the nutritional content of food; the absorption, bioavailability and metabolism of nutrients; and/or disrupt the balance of nutrients that could result in adverse nutrient/nutrient interactions. Although many nutritional components will be studied, the emphasis will be on mineral nutrients.
Human feeding studies using Western-type diets will evaluate the benefits of foods modified to enhance the concentration and chemical forms of selenium, for improving immune and neuropsychological function, antioxidant defense, and cancer risk. Cellular, animal, and molecular biology methods will be used to assess how the food forms of selenium affect its nutritional utilization and distribution into selenoproteins. The effect of common dietary variables on iron and zinc bioavailability and requirements will be assessed in human and animal studies. Human studies will assess iron bioavailability from plant-based diets with reduced meat content, as influenced by control of heme and nonheme iron absorption, iron excretion, and human genotype. Human studies will assess the efficacy of heme iron supplements and of elemental iron fortificants for improving iron status. Further human studies will test the impact of high phytic acid content of or calcium fortification of foods on zinc and iron absorption. Human intestinal cell culture models and transgenic animal models will be used to determine how newly identified transport proteins for iron, zinc, and copper regulate the absorption of and interactions between these nutrients. Cellular and animal studies will evaluate the benefits of minor crops grown in the Northern Plains areas, such as buckwheat and flax, for improving mineral intake, altering lipid concentration, increasing antioxidant status, and decreasing the risk of cancer and diabetes.

2. **How serious is the problem? Why does it matter?**
This research will help determine food and diet patterns that provide the amounts and chemical form(s) of nutrients needed to complement our genetic potential for good health. Identification of the forms and food sources of selenium compounds that influence immune, neuropsychological, antioxidant, and anticarcinogenic function will facilitate evidence-based dietary recommendations to improve health and reduce disease risk. Investigation of the bioavailability of iron from food combinations and from different fortification or supplemental forms of iron is needed to effectively reduce iron deficiency anemia, the most common nutrient deficiency in the world, that impairs reproduction, cognitive development, and physical work capacity, and to avoid excessive iron nutriture that may be associated with

oxidative diseases in some segments of Western societies. Improving the understanding of trace element interactions will provide needed guidance for supplementation and fortification of foods to meet nutritional needs while reducing the risk of absorption and retention of toxic minerals. Research on dietary mineral bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public. Studies of minor dietary constituents, such as buckwheat, that may provide the substrate for the formation of chiro-inositol, a putative insulin mediator, and be a good source of mineral nutrients, are needed to show how an increase in dietary diversity might provide protection against chronic disease.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
This project is part of National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%). It is directly related to Human Nutrition Performance Goal 3.1.1 Human Nutrition Requirements and 3.1.3 Nutritious Plant and Animal Products, concerning the priority objective: Bioavailability of Nutrients and Food Components. It is in the same management unit and is coordinated with CRIS 5450-51000-031-00D, "Role of Dietary Minerals on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk", especially in relation to Se-containing food components with possible cancer preventive properties. It also coordinates with CRIS 5450-51000-034-00D, (Mineral Intakes for Optimal Bone and Joint Development and Health), in measuring calcium bioavailability from diets.

4. **What were the most significant accomplishments this past year?**
A. Reduced iron requirements and susceptibility to excess dietary iron may be especially likely in the 10% of the non-hispanic white US population that carry the genetic mutation associated with hemochromatosis, a disorder of excess iron accumulation. At the Grand Forks Human Nutrition Research Center, 11 volunteers who carry this mutation (HFE C282Y heterozygotes) had their iron absorption measured from a hamburger meal with or without extra fortification with iron and vitamin C. Compared with controls, subjects who carry the mutation did not absorb significantly more of either heme (from meat) or nonheme forms of iron from these meals. These results indicate that the fortification of foods with iron and ascorbic acid is not likely to adversely affect body iron accumulation by individuals carrying the genetic mutation for hemochromatosis.

B. Dietary recommendations for zinc have been difficult to determine because there are no generally accepted indicators of marginal zinc status in humans. At the Grand Forks Human Nutrition Research Center, this problem was addressed by measuring people(s ability to adapt their absorption of zinc from diets differing in zinc content. After 4 wk, adaptation to meet the homeostatic requirements of healthy US men and women did not appear necessary if low-phytate diets contained at least 5.4 to 7.7 mg/2500 kcal. Within 4 wk of consuming a controlled diet, healthy US adults adapted to absorb zinc more efficiently if their diet contained less than 4.5 mg Zn, but apparently did not need to adapt if the diets contained more zinc, up to 20 mg zinc daily. Adaptation in zinc absorption will be explored further as a possible criterion for determining human zinc requirements.

Marginal intakes of the essential mineral nutrients zinc, iron, and calcium enhance the absorption of cadmium and stimulate the accumulation of cadmium in the upper small intestine (duodenum) of the rat model used to study mineral metabolism in humans. A study was done at the Grand Forks Human Nutrition Research Center to determine the physiological mechanism for this phenomenon by using the Caco-2 cell, a model of the human intestinal cell in culture. It was found that an increase in the concentration of calcium in the media used to feed the cells, reduced cadmium

accumulation in the cell and cadmium transport across the cell layer, while high zinc or iron, or low iron had no effect on cadmium accumulation or transport. These findings suggest that the human intestinal cell acts differently than the rat intestinal cell during mineral absorption, and cast doubt on the use of the rat to study absorption phenomena in humans, or suggests that the Caco-2 in vitro system is not a good model for the study of in vivo mineral uptake and transport mechanisms.

In the laboratory rat model, zinc deficiency causes a dramatic reduction in food intake, which is thought to affect the self-selection of the amount of fat, protein, and carbohydrate in the diet when the rat is given a choice of multiple diets with different concentrations of these nutrients. A study was done at the Grand Forks Human Nutrition Research Center where computerized feeding machines were used to monitor the minute-by-minute food intake over a 24-hr period. It was found that the food intake patterns were similar among zinc deficient and control rats, but the zinc deficient rat ate less food, while at the same time it self-selected a diet that contained less protein, a higher amount of carbohydrate, but no change in the amount of fat. These findings suggest that this model would be a good one to study the biochemical mechanisms of food intake regulation, which could help solve the mystery of anorexia and/or over-eating in humans.

A physiological phenomenon that is not understood is why copper deficiency causes iron deficiency in most animal species. A study was carried out at the Grand Forks Human Nutrition Research Center to determine the effects of copper deficiency on various blood parameters usually associated with iron deficiency in rats. By using a state-of-the-art technique called flow cytometry, it was shown that both copper and iron deficiencies individually caused anemia and induced the release of immature red cells, included nucleated ones, from the bone marrow into the blood, but when both deficiencies were present together, the effects were much stronger. This plus other findings in the study suggest that copper deficiency reduces the activity of a previously unknown copper-dependent iron oxidase required for proper incorporation of iron into developing red cells, and showed that flow cytometry is the technique of choice in the study of copper/iron interactions in blood cell formation.

Copper deficiency causes iron deficiency in most animal species and part of the effect is thought to be the inhibition of a copper-dependent iron oxidase (Hephaestin) in the intestinal cells that is required to oxidize iron before it can be absorbed. A study was carried out at the Grand Forks Human Nutrition Research Center to determine whether copper deficiency reduces the absorption of iron by the intestine. By using a test diet labeled with radioactive iron and using whole-body-counting techniques, it was shown that copper deficiency actually enhanced iron absorption instead of reducing it as expected. Although a genetic disease in animals that blocks Hephaestin activity causes the animal to become anemic, this study shows that there must be some other mechanism for iron absorption that bypasses this oxidation step in copper deficient animals.

Manganese is an essential nutrient that can also be toxic at high doses; although simple manganese toxicity only occurs at very high intakes, toxicity may also occur at lower intakes by interaction with other dietary components. A study was conducted at the swine facility at North Dakota State University (collaboration with Dr. Joel Caton, Department of Animal Science) to determine whether marginal deficiency of magnesium interacted with moderately high intakes of manganese to predispose swine to death by ischemic heart disease. Electron micrographs of heart mitochondria from forty eight swine fed (in a crossover design) diets either adequate or deficient in magnesium and adequate or high in manganese showed that

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high manganese intakes combined with magnesium deficiency resulted in severe ultra-structural damage to the mitochondria. This study showed that intakes of manganese considered safe under typical production conditions may result in severe impairment of health when combined with other nutritional stressors, such as magnesium deficiency.

C. None

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
This project has continued investigations of various aspects of copper, iron, selenium and zinc absorption and metabolism. Some highlights include:
- Marginal intakes of zinc, iron, and calcium greatly enhanced the accumulation of cadmium in the upper small intestine, leading to a higher accumulation of the toxic metal in the liver and kidneys, which suggests that populations with these nutrient deficiencies are especially susceptible to cadmium toxicity.
- A novel role for selenium in the up-regulation of cell cycle related genes was discovered, that may lead to a better understanding of the essentiality of selenium as a nutrient and its involvement in cancer prevention.
- Although Se-methyl selenocysteine was the primary form of selenium in broccoli, methyl selenol was the primary form of Se absorbed by intestinal cells in culture, which may help explain the observed high efficacy of Se from broccoli for preventing colon cancer.
- Elemental iron powders commonly used to fortify staple foods with iron were only 21-64% as bioavailable to rats as iron from ferrous sulfate, suggesting that higher concentrations of these forms may be needed if they are used in international iron fortification programs.
- In a sample of 262 healthy premenopausal US women, body iron stores were primarily predicted by a self-estimation of menstrual losses, and less substantially related to the dietary consumption of meat, phytic acid (from whole grains, legumes, and nuts), tea, or iron supplements, suggesting that low iron stores in many US women may be primarily related to increased menstrual loss, rather than poorer diets.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
Year 1: Determine the bioavailability to humans of two elemental iron powders commercially produced to fortify cereal grains, and test how the absorption of elemental electrolytic iron powder is affected by interactions with ingested ascorbic and phytic acids.

Determine the influence of micronization and encapsulation of ferric pyrophosphate, an iron form used in food fortification, on iron bioavailability to rats.

Compare conventionally-grown broccoli to organically-grown broccoli and determine whether production system has more of an impact on the nutritional profile than other environmental variables such as climate, water usage and season.

Characterize the phenolic acids in broccoli and determine whether they are associated with some of the anti-oxidant properties of broccoli.

Produce antibodies to the divalent metal transporter 1 (DMT1) and iron transporter ferroportin 1 (FNP1) for studying their relationships to copper deficiency and related interactions with iron uptake and transport in both animal and cell culture models.

Use the metallothionein knockout mouse to determine the relationship between

intestinal metallothionein and cadmium accumulation in the duodenal enterocytes.

Produce antibodies to intestinal zinc transporters, Znt1 and ZIP2, proteins to aid in animal and human cell culture studies of how the concentration of dietary zinc regulates its own rate of intestinal absorption.

Year 2: Measure iron excretion rates for women, based on isotopic dilution over a 3-year period, and relate iron excretion to body iron stores.

Determine whether humans adapt to moderate the absorption and retention of supplemental zinc.

Conduct a supplementation trial in humans to determine the bioavailability of selenium from high-selenium beef.

Work with food companies to develop and test high-Se foods made from agricultural products grown on high-selenium soils in the Dakotas and California.

Determine the relative bioavailability of selenium and zinc from buckwheat farinetta using the rat as a model.

Year 3: Compare the bioavailability of commonly used elemental iron fortification powders, using sensitive isotope retention measurements in humans.

Further assess measurements of adaptation in zinc absorption from controlled diets as a physiological criterion for determining human zinc requirements.

Determine whether interactions between selenium, plant fiber and phytochemicals affect the biological actions of selenium such as incorporation into selenoproteins and protection against colon cancer.

Determine relationships among copper deprivation, iron absorption and ferroxidase (Hephaestin) activity in the intestine of the rat model.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
Cooperated with a non-profit charitable organization to share research results on the bioavailability of elemental iron powders to anemic rats, as part of a larger project to assess these products by a variety of methods, concluding with human efficacy trials.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
PG Reeves gave a presentation on (Food Cadmium Bioavailability: A Rediscovery of Old Facts) to the UND Sigma Xi Chapter Meeting, Grand Forks, ND, September, 2002.

PG Reeves gave a presentation on "Bioavailability of Food Cd as an Issue in Risk Management and Assessment" at the SCOPE Environmental Cadmium Workshop, Ghent, Belgium, September 4, 2003.

JR Hunt gave a presentation on "Contributions of AIN women to vitamin and mineral research" American Society of Nutritional Sciences (Experimental Biology) meeting,

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San Diego, CA, April 20, 2003.

Rosalie Marion Bliss wrote an article (Testing the Fortitude of Iron in Cereals) for Agricultural Research magazine, May, 2003, p. 15, about our research on the bioavailability of elemental iron powders.

John Finley gave a presentation on "Supplementing selenium intake with high-selenium foods" to the Chinese Center for Disease Control in three Chinese provinces.

John Finley gave a presentation "Supplementing dietary selenium with high-selenium vegetables" to the Crop and Food division of the Crown Research Institute in Palmerston North, New Zealand.

Nutriview 2003, issue 2, (http://www.nutrivit.org/vic/staple/index.htm) published a brief report, (Particle surface area may predict bioavailability of iron fortificants), about our research on the bioavailability of elemental iron powders.

Food Navigator.com (http://www.foodnavigator.com/news/news.asp?id=7687) published a brief report, (Stick to the spinach?), about our research on the bioavailability of elemental iron powders, 5/26/03.

Publications: Log 115:

1. J. W. Finley. The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. Nutrition Reviews. 2003. 61 (7):250-254. 0000144908
2. J. Finley, J. Penland, R. Petit, C. Davis. Dietary Manganese Intake and Type of Lipid Do Not Affect Clinical or Neuropsychological Measures in Healthy Young Women. Journal of Nutrition. 2003. 133 (9) p. 2849-2862. 0000134428
3. Roughead, Z.K., Zito, C.A., Hunt J.R. Initial uptake and absorption of nonheme iron and absorption of heme iron in humans are unaffected by the addition of calcium as cheese to a meal with high bioavailability. American Journal of Clinical Nutrition. 2002. 76:419 425. 0000121210
4. Hunt, J.R., Johnson, L.K., Juliano, B.O. Bioavailability of zinc from cooked Philippine milled, undermilled, and brown rice as assessed in rats by using growth, bone zinc and zinc 65 retention. Journal of Agricultural Food Chemistry, 2002. 50:5229 5235. 0000134420
5. Hunt, J.R. Tailoring advice on dietary supplements: An opportunity for dietetics professionals. J American Dietetic Association. 2002. 102:1754 1755. (Invited Commentary). 0000140266
6. Swain, J.H., Hunt, J.R. Carbonyl iron is a more effective hemoglobin repletion agent than electrolytic of reduced iron powder in rats. Proceedings of the North Dakota Academy of Science. 2002. 56:59 (Short technical communication). 0000134373
7. Hunt, J.R., Roughead, Z.K. Animal models of Human Nutrition. In Encyclopedia of Food Sciences and Nutrition, 2nd Ed. Eds: Caballero B, Trugo L, and Finglas P. London, Academic Press. 2003. pp. 232 238. (Encyclopedia paper). 0000112093
8. Reeves, P.G. Patterns of food intake and self-selection of macronutrients in rats during short-term deprivation of dietary zinc. Journal of Nutritional Biochemistry. 2003. v. 14. p. 232-243. 0000139169
9. Swain, J.H., Hunt, J.R. Elemental iron powder used for food fortification: does physicochemistry predict bioavailability? International Nutritional Anemia Consultative Group (INACG) Symposium, February 6, 2003, Marrakech, 0000135852

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Morocco. Abstract #95, p. 41.

10. Hunt J.R., Johnson L.K. Self reported menstrual losses predict iron status in premenopausal U.S. women. International Nutritional Anemia Consultative Group (INACG) Symposium, February 6, 2003, Marrakech, Morocco. Abstract #55, p. 30. 0000135855

11. Hunt, J.R., Johnson, L.K. Adaptation in zinc absorption from whole diets. International Zinc Nutrition Consultative Group (IZiNCG) Symposium, February 7, 2003, Marrakech, Morocco. Abstract #10. 0000144725

12. Hunt, J.R., Johnson, L.K. Adaptation in dietary zinc absorption from whole diets: Implications for determining zinc requirements. FASEB Journal. 2003. v. 17:A694. 0000141824

13. Roughead, Z.K.R., Zito, C.A., Hunt, J.R. Dietary calcium (Ca) inhibits the intestinal uptake, not transfer, of heme iron (Fe) and reduces total Fe retention from a typical Western meal. FASEB Journal. 2003. v. 17:A1086. 0000141860

14. Swain, J.H., Hunt, J.R. The bioavailability of elemental iron powders used for food fortification varies among commercial forms and is influenced by physiochemistry. FASEB Journal. 2003. v. 17:A1132 3. 0000141829

15. Hunt, J.R., Zeng, H. Heme and nonheme iron absorption in humans with the C282Y and H63D mutations associated with hemochromatosis. BioIron 2003 Symposium, May 4 9, 2003, Washington, D.C. Abstract # 62, p. 166. 0000146467

16. Reeves, P.G., Ralston, N.V.C., Idso, J.P., Lukaski, H.C. Dietary Cu-Fe-amino acid interactions: flow cytometry of rat hematology indices. FASEB Journal. 2003. v. 17(5): Abstract p. A1128. 0000141332

17. Reeves, P.G., Chaney, R.L. Marginal intakes of zinc, iron, and calcium enhance the accumulation of cadmium in the intestine of rats fed a rice-based diet. FASEB Journal. 2003. v. 17(5): Abstract p. A1163. 0000141338

18. Chaney, R.L., Reeves, P.G. Food-Chain Transfer and Bioavailability of Cd from Soils and Soil Amendments. American Chemical Society. 2002. Abstract. 0000135454

19. Chaney, R.L., Ryan, J.A., Reeves, P.G., Simmons, R. A new paradigm for environmental cadmium risk assessment. Scientific Program of the 2nd Conf. Molecular Mechanisms of Metal Toxicity and Carcinogenesis; Morgantown, WV.). NIOSH, Morgantown, WV. 2002. Abstract. p. 33. 0000138089

20. Reeves, P.G., Chaney, R.L. Mineral nutrient status affects the bioavailability of cadmium in food. Scientific Program of the 2nd Conf. Molecular Mechanisms of Metal Toxicity and Carcinogenesis. NIOSH, Morgantown, WV. 2002. Abstract. p. 89. 0000136962

21. Chaney, R.L., Ryan, J.A., Reeves, P.G., Kukier, U. Limited phyto- and bio-availability prevent risk from cadmium in regulated biosolids. Abstracts of the Society for Environmental Toxicology and Chemistry Annual Meeting. 2002. Abstract No. 164. 0000135465

Approved: ROOS ERIC E **Date:** 09/26/2003

Project Number: 5450-51000-032-04T **Accession:** 0402795 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF FOOD FORMS OF SELENIUM

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
None.

2. **How serious is the problem? Why does it matter?**
None.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
None.

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2003:
An initial study with a breakfast cereal product made from high-selenium wheat provided evidence that selenium from wheat cereal was effective for reduction of colon cancer; however the methionine content of a diet may affect selenium metabolism and potential efficacy of a source of selenium against cancer. To characterize better the ability of selenium form wheat cereals to protect against colon cancer, rats treated with a chemical carcinogen were fed diets containing cereal from wheat high and low in selenium, but the methionine content of the diet was slightly below adequate. Contrary to results of the first study, lesions indicative of colon cancer were not affected by selenium in the present study, and the lack of an effect may have been a result methionine insufficiency diverting selenomethionine from wheat out of anticancer pathways. These data show that the health benefits of selenium from wheat may be partially dependent on amino acid (especially methionine) status of the animal.

D. Progress Report:

This report serves to document research conducted under a Cooperative Research and Development Agreement (CRADA), #58-3K95-0-813, between ARS and General Mills, Inc. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

The purpose of this CRADA is to study whether high-Se wheat from high-Se areas of the Northern Great Plains that has been converted into a whole wheat cereal product can be used to safely and effectively provide supplemental Se to humans with adequate Se status.

The ARS is conducting human and animal studies and analyzing all samples collected during the experiment. General Mills, Inc. is converting high-Se wheat into a ready-to-eat cereal product.

Publications: **Log 115:**

Project Number: 5450-51000-032-04T **Accession:** 0402795 **FY:** 2003

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-05T **Accession:** 0403407 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: HIGH SELENIUM MEAT, WHEAT, AND BROCCOLI: A MARKETABLE ASSET?

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2002: The biological actions of selenium are strongly dependent on its chemical form. Because different foods contain different chemical forms of selenium, it is hypothesized that selenium from different foods will undergo different metabolic transformations and result in different biological outcomes. High-selenium broccoli and meat (pork) were labeled with radioactive selenium and fed to rats; animals were killed, dissected and the amount of radioactive selenium in tissues and specific selenoproteins was determined. Selenium from pork was retained better than broccoli selenium, and retention was dependent on dietary selenium concentrations; selenium from pork readily incorporated into selenoproteins but selenium from broccoli was undetectable in selenoproteins. These results show that selenium from meat goes into a pool more easily incorporated into proteins and retained in tissues; selenium from broccoli goes into a more labile pool and that pool may be associated with the previously observed efficacy of high-selenium broccoli for protection against colon cancer.

B. Other Accomplishments: High-selenium beef, wheat and broccoli all are excellent sources of supplemental dietary selenium, but the bioavailability of selenium from these foods has not been determined. Rats were fed diets containing selenium concentrations of 0.001 to 2 parts/million, and the selenium was supplied as high-selenium beef, wheat or broccoli. Tissue selenium levels, selenoprotein activities and selenoprotein mRNA concentrations were all altered to different degrees depending on the tissue and the food source of selenium, but in general selenium from high-selenium beef was the most bioavailable. These results demonstrate that the bioavailability of selenium from selenium-enriched foods depends on the specific food, and bioavailability should be taken into account when promoting a food as a source of supplemental selenium.

C. Special populations: None

D. Progress Report: This report serves to document research conducted under a Trust Fund Agreement between ARS and the Initiative for Future Agricultural and Food Systems (IFAFS), CREES/USDA. In addition to work conducted at the GFHNRC, money from this grant has been transferred to the following projects: 5450-51000-032: -06S, -07G, -08S, -09S, -010G, -11S. For complete details of work accomplished under this project, annual reports detailing work conducted in the above agreements also should be consulted. Additional details of this research can be found in the report for the parent CRIS 5450-51000-032-00D.

Publications: Log 115:

Project Number: 5450-51000-032-05T **Accession:** 0403407 **FY:** 2003

1. Hintze,K.J., Wald, K., Zeng, H., Jeffery, E., Finley, J. Thioredoxin Reductase in Human Hepatoma Cells Is Transcriptionally Regulated by Sulforaphane and Other Electrophiles Via an Antioxidant Respønse Element. Journal of Nutrition. 2003. 133 (9) 2721-2727. **0000144726**
2. Bañuelos, G.S., Vickerman, D.B., Trumble, J.T., Shannon, M.C., Davis, C.D., Finley, J.W., Mayland, H.F. Biotransfer possibilities of selenium from plants used in phytoremediation. International Journal of Phytoremed. 2002. 4: 315-329. **0000132721**
3. Finley, J.W., Grusak, M.S., Keck, A.S., Gregoire, B.R. Bioavailability of selenium from meat and broccoli as determined by retention and distribution of Se75. FASEB Journal. 2003. v.17: A1136. **0000141800**
4. Hintze, K.J., Wald, K.A., Jeffery, F.H., Finley, J.W. Transcriptional induction of thioredoxin reductase (TR). FASEB Journal. 2003. v. 17: A1371. **0000141806**
5. Hintze, K.J., Belgarde, S., Jeffery, E.F., Finley, J.W. The effect of dietary sulforaphane (SF) and selenium (Se) on thioredoxin reductase (TR), glutathione peroxidase (GPX) and quinone reductase (QR) activities in male Fisher-344 rats. Functional Foods for Health Meeting. 2003. **0000150544**
6. Hintze, K.J., Keck, A.S., Finley, J.W., Jeffery, E.H. Induction of hepatic thioredoxin reductase activity by sulforaphane, both in Hepa1c1c7 cells and in male Fisher 344 rats. Journal of Nutritional Biochemistry. 2003. 14 (3):173-179. **0000135858**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-06S **Accession:** 0404307 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: AGRICULTURAL PRODUCTION ASPECTS OF HIGH SELENIUM MEAT AND WHEAT

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

2. **How serious is the problem? Why does it matter?**

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2003:

Beef is one of the most important sources of selenium in the North American diet, and feeding beef cattle high-selenium feedstuffs increases the selenium content of muscle tissue; the impact of short-term feeding of high-selenium diets on performance of beef animals in a feedlot has not been evaluated. A series of studies fed growing beef steers diets composed of selenium-enriched forages and grains; animals were slaughtered and carcass and performance traits were assessed. Diets containing up to 10 parts/million selenium did not alter carcass characteristics or feedlot performance measures such as feed efficiency and rate of gain. These results demonstrate that beef cattle in a feedlot can be safely and reliably enhanced in selenium concentrations without adversely affecting economically important production traits.

B. Other Accomplishments:

Beef cattle may accumulate selenium in edible muscle when they are pastured on high selenium forages, but the effect of seasonal change on bioavailability of selenium to cattle has not been evaluated. Forage samples from multiple sites on a ranch with a history of selenium toxicity were obtained at times from May until September; samples were collected by hand as well as by fistulated cattle and the experiment was repeated for two consecutive seasons. Selenium concentrations rose initially then fell as season progressed; cattle tended to select forage of better quality and higher selenium content than the average obtained by clipping all plant material. These results will help cattle producers devise grazing strategies that maximize the intake of trace elements, especially selenium.

D. Progress Report:

This report serves to document research conducted under a Specific Cooperative Agreement, #58-5450-1-310, between ARS and North Dakota State University. Additional details of the research can be found in the report for the parent CRIS

Project Number: 5450-51000-032-06S **Accession:** 0404307 **FY:** 2003

5450-51000-032-00D.

Dr. J. Caton was a co-author on the grant that has supplied the money for this project. The overall project is doing a comprehensive study of how high-Se wheat, meat and broccoli are produced and utilized by animals. Dr. Caton is conducting the Animal Science portion of the project.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-07G **Accession:** 0404348 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: CHEMICAL FORMS OF SELENIUM IN FOODS

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
The biochemical actions of selenium depend on the chemical transformations of selenium metabolites after they are consumed; there is little information regarding such transformations in the gut. High-Se broccoli was subjected to simulated gastric and intestinal digestion, and the digestate was applied to the apical side of a monolayer of gut cells in culture (CACO-2); the chemical forms of Se in the digestate were determined before and after it exposure to CACO-2 gut cells. Upon exposure to the cells, methylated forms of Se such as Se-methyl selenocysteine were primarily converted to mono-methyl selenol. These results show that cells in the gut immediately convert some forms of selenium in food to simpler forms even before they are absorbed, and such transformations must be taken into account when determining the biological efficacy of a certain form of selenium for a particular outcome (such as cancer protection).

B. Other significant accomplishments:
None.

C. Special populations:
None

D. Progress Report
This report serves to document research conducted under a Grant-type Agreement between ARS and the Department of Chemistry, University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.
The purpose of this Trust is to study the chemical speciation of Se in foods, and relate those forms of Se to the observed health benefits of Se from foods. Mass spectrometric methods will be developed to determine the forms of Se in broccoli, wheat and beef grown under different production conditions.

Publications: **Log 115:**

1. Roberge, M.T., Borgerding, A.J., Finley, J.W. Speciation of selenium compounds from high selenium broccoli is affected by the extracting solution. Journal Agricultural Food Chemistry. 2003. 51: 4191-4197. 0000144731
2. Roberge, M, Borgerding, A., Finley, J. Specific selenium species from broccoli are transported differently across a Caco-2 cell monolayer. FASEB Journal. 2003. v. 17: A1136. 0000141803
3. Roberge, M., Finley, J.W., Borgerding, A., Lukaski, H.C., Kozliak, E. Advantages of the pulsed discharge helium ionization detector (PDHID) in detecting separations of bacterial metabolites. Symposium on Capillary Chromatography and Electrophoresis. 2003. 0000145610

Project Number: 5450-51000-032-07G **Accession:** 0404348 **FY:** 2003

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-08S **Accession:** 0404351 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF HIGH-SELENIUM FOODS TO HUMANS

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

2. **How serious is the problem? Why does it matter?**

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**

4. **What were the most significant accomplishments this past year?**
   D. Progress Report:

   This report serves to document research conducted under a Specific Cooperative Agreement, #58-5450-1-315, ARS and Oregon State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

   Dr. Whanger has a long-standing interest in Se metabolism, and especially how different forms of Se affect the use and distribution of Se in the body. Dr. Whanger was a co-author of the IFAFS grant award that is being used to pay this Specific Cooperative Agreement and Dr. Whanger has been instrumental in putting together the research agreement with the Chinese Academy of Preventive Medicine (#5450-51000-032-11S).

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-09S **Accession:** 0404746 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF INTERACTING PHYTOCHEMICALS IN BROCCOLI

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
A chemical found in broccoli, sulforaphane, may be protective against cancer by functioning as an anti-oxidant and reducing oxidative stress with a subsequent reduction in DNA damage. Cells grown in the presence or absence of sulforaphane were subjected to chemically-induced oxidative stress and damage to DNA was assessed by the COMET assay (a measure of DNA single strand breaks). Sulforaphane treatment resulted in decreased incidence of DNA strand breaks. These results indicate that some of the health benefits of broccoli may be a result of the antioxidant activity of sulforaphane preventing DNA damage.

B. Other significant accomplishments: None.

C. Special populations: None.

D. Progress Report
This report serves to document research conducted under a Specific Cooperative Agreement between ARS and Department of Nutritional Sciences, University of Illinois. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.
The purpose of this Agreement is to study the nutritional benefits of broccoli consumption, concentrating especially on phytochemicals such as sulforaphane. Human and animal studies are being conducted at the GFHNRC, phytochemical analyses are being conducted at the University of Illinois. Additionally, a visiting scientist from Univ. Ill is working at the GFHNRC and is helping conduct the human and animal studies.

Publications: Log 115:

1. Hintze, K.J., Keck, A.S., Finley, J.W., Jeffery, EH. Induction of heptic thioredoxin reductase activity by sulforaphane, both in Hepalclc7 cells and in male Fisher 344 rats. Journal of Nutritional Biochemistry. 2003. 14:173-179. **0000135858**
2. Keck, A.S., Finley, J.W. Selenium (Se) enriched beef, wheat or broccoli or the salt selenate differ in the ability to increase thioredoxin reductase (TR) and glutathione peroxidase (GPx) in rats. FASEB Journal. 2003. v.17: A1136. **0000141797**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-10G **Accession:** 0404556 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: USE OF GEOLOGICAL INFORMATION SYSTEM TO VISUALIZE HIGH SELENIUM SOIL AND CROPS

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
None.

2. **How serious is the problem? Why does it matter?**
None.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
None.

4. **What were the most significant accomplishments this past year?**
A. Factors affecting the bioavailability of selenium from the soil to plants are not well understood. A study conducted on a ranch (a location known to have high concentrations of Se in the soil) in Central South Dakota (in collaboration with the Department of Geology, University of North Dakota) examined geological factors in the soil and related them to selenium accumulation in plants grown in close proximity. Selenium in plants was not related to total selenium concentrations in soil, but was instead primarily associated with soluble soil Se and factors that affect the reducing environment of the soil. These data show that producers that wish to grow high selenium crops can not rely on simply having soils high in selenium, but instead the soils must be of a chemical composition that promotes the formation of bioavailable selenium

B. None
C. None

This report serves to document research conducted under a Grant Agreement, #58-5450-1-325, between ARS and the University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

The purpose of this project is to examine the geological, geographical and soil-related factors that result in the development of high-Se soils and the subsequent production of high-Se crops. Knowledge of these factors is essential if producers ever attempt to market high-Se wheat on a regular basis.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-11S **Accession:** 0404830 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: SELENIUM SUPPLEMENTATION OF SUBJECTS WITH EXTREMELY LOW SELENIUM INTAKES

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2003: Study in progress, no accomplishments to report.

D. Progress Report:

This report serves to document research conducted under a Specific Cooperative Agreement, #58-5450-1-F-163, between ARS and the Chinese Academy of Preventive Medicine in Beijing, China. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

An overall objective in our laboratory is to determine whether foods enriched in Se can safely and effectively be utilized as supplemental sources of Se. To this end we are conducting field studies that are examining the geological and biological processes that cause accumulation of Se in soil, plants and animals. We also are conducting basic nutritional trials in animals that are determining whether Se from wheat, meat and broccoli is effective for a number of health-related problems such as prevention of colon cancer. The ultimate measure of the "usefulness" of these food sources of Se is to feed them to a target population and measure basic aspects of health and metabolism.

This project is feeding high-Se broccoli, wheat and meat to healthy humans living in a low-Se area of China. We have chosen to study this area of China because Se intakes there are the lowest in the world.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-13T **Accession:** 0405631 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: COMBATING IRON DEFICIENCY: ABSORPTION & EFFICACY IN HUMANS OF ELEMENTAL IRON POWDERS & HEME IRON

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. None.

B. None.

C. None.

D. This is a report of research conducted under the USDA ARS CRIS project 5450-51000-032-00D, concerning the USDA CSREES Nutritional Research Initiative competitive grant 2002-01885 (CREES CRIS 0192629) entitled "Combating Iron Deficiency: Absorption and Efficacy in Humans of Elemental Iron Powders and Heme Iron". This project has the following two objectives: 1) To determine the efficacy of fortifying food with elemental iron powders, relative to equivalent amounts of ferrous sulfate, or of supplementing with a limited amount of iron in the heme form, on serum ferritin in women with low iron stores. Supporting objectives include assessment of the effect of these iron sources on: absorption of nonheme iron from other dietary sources, oxidative stress in the lower intestine, plasma zinc, attention, and affect (mood). 2) To determine the absorption of irradiated electrolytic iron powder, relative to ferrous sulfate, as affected by dose and by interactions with ascorbic acid and phytic acid. This year funding was received. Approved human studies protocols were completed for 52 subjects for objective 1 and an additional 56 subjects for objective 2. Chemical analyses of the data are in the final stages of completion, and statistical analysis of the data has begun. The results are not yet ready for reporting. Completion of data analysis and reporting for the first objective is expected within the next year. This research will help establish the efficacy of elemental iron powders used in food fortification for improving human iron deficiency, a global nutrition problem. This year funding was received, subjects were successfully recruited for studies addressing both objectives and biological samples were collected for further chemical and statistical analysis. Results are not yet available. Completion of data analysis and reporting for the first objective is expected within the next year. This research will help establish the efficacy of elemental iron powders used in food fortification for improving human iron deficiency, a global nutrition problem.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-14T **Accession:** 0405700 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: CHANGING THE NUTRITIONAL COMPOSITION OF BEEF: CAN WE MITIGATE THE RISK OF COLON CANCER?

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
A human study that will feed diets containing un-enriched or enriched in selenium is planned for 2004, but the study requires that meat high and low in selenium be produced and processed. High-Se beef was produced (collaboration with Martin Marchello, North Dakota State University) by feeding high-Se diets and low-Se beef was obtained by buying cattle from a low-Se region on the East coast; all animals were slaughtered and ground into lean ground beef. Trial menus showed that the beef product was suitable for human consumption and a trial rat study showed that it could be incorporated into rat diets. Production of this meat will allow full scale human and animal trials examining the benefits of consuming selenium-enriched meat to be conducted.

B. Other significant accomplishments:
Although selenium supplementation is known to decrease the risk of several cancers (including colon cancer), it is unknown whether Se from beef is effective for the suppression of colon cancer. An initial study of the benefit of Se from beef for the suppression of colon cancer was conducted in rats fed diets containing either high or low-Se beef; cancer was induced with a chemical carcinogen. There was no difference in colon cancer incidence (as assessed by counting pre-neoplastic lesions called aberrant crypts) between high and low Se beef. Although these results need to be confirmed, they do not support the hypothesis that Se from beef is effective for the suppression of colon cancer.

C. Special populations:
None

D. Progress Report
This report serves to document research conducted under a Trust Agreement between ARS and the National Cattlemen's Beef Association. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.
The purpose of this Trust is to study whether high-Se beef produced in high-Se areas of the Northern Great Plains can be used to safely and effectively provide supplemental Se to humans with adequate Se status. Animal studies will determine if Se from high-Se beef is effective for prevention of colon cancer.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-16S **Accession:** 0405998 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS TO SUPPORT RESEARCH ON HUMAN MINERAL NUTRITION

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
None.

2. **How serious is the problem? Why does it matter?**
None.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
None.

4. **What were the most significant accomplishments this past year?**
D. This report serves to document research conducted under Specific Cooperative Agreement #58-5450-2-335 between ARS and University of North Dakota. Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-032-00D and related CRIS project 5450-51000-034-00D.

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, applying an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

Accomplishments this year included health physicist support of investigations to assess bioavailability of calcium, iron, and zinc using gamma-emitting radiotracers in human absorption studies. The health physicist led the work of the FDA-sanctioned UND Radioactive Drug Research Committee, which reviewed and provided oversight for all nutrition experiments employing radioactive tracers. An evaluation of over 10 years of human whole body counting data indicated that body activity of bismuth-214 (a decay product of radon) was greatest in the fall quarter and least in the spring quarter. This seasonal variation is consistent with increased body radon accumulation in enclosed buildings, and possibly increased body fat (sites of

Project Number: 5450-51000-032-16S **Accession:** 0405998 **FY:** 2003

physiological radon storage) during winter months. In the coming year, additional work will evaluate scintillation counting of Ca-47 in the calcaneus (heel), as a complement to whole body counting analyses. These accomplishments support the sensitive detection of gamma-emitting isotopic tracers, providing uniquely sensitive measurements of mineral retention in humans, as affected by nutritional status, dietary sources of nutrients, and the human genotype.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
None.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
None.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
Reports authored by cooperators (non-ARS scientists):

Momcilovic, B., Lykken, G.I. Distribution of 210Po and 210Bi radon daughters in the brain proteins of a subject who suffered from Alzheimer¿s Disease. V. simpozij HDZZ, Stubicke Toplic. 2003. p. 259-263.

Lykken, G.I., Momcilovic, B. Myelin fat storage of environmental radon daughters in the etiology of multiple sclerosis - a new approach. North Dakota Academy of Science, March 27, 2003, Minot, ND.

Lykken, G.I., Momcilovic, B. Whole body counting of scandium-47 gamma emission extends in-vivo human and animal calcium-47 metabolic studies for additional two weeks and more. Experimental Biology 2003, April 1-15, 2003, San Diego, CA.

Lykken, G.I., Momcilovic, B. Environmental radon, high energy alpha particle radiation, and multiple sclerosis connection revisited. Health Physics Society, 48th Annual Meeting, July 21-23, 2003, San Diego, CA.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-17T **Accession:** 0406927 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH

National Programs: 107 100% Human Nutrition

Title: NUTRITIONAL VALUE OF BUCKWHEAT: TRACE ELEMENT VARIABILITY AND BIOAVAILIBILITY AND FAGOPYRITOL CONTENT

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
Buckwheat may be an excellent source of several nutritionally important chemicals, including trace elements such as selenium, but sources of variation in the trace element content of field grown buckwheat have not been characterized. In a study conducted at multiple sites across North Dakota (collaborative with MInDak Growers, Ltd., Grand Forks, ND) harvested samples of buckwheat (as well as soil samples from the same field) were collected from fifty buckwheat producers. Analysis showed that all buckwheat samples were average to high in selenium (compared to other grains), but concentrations were variable and were primarily affected by geographic location (soil selenium concentrations were not well associated with buckwheat selenium concentrations). These results show that depending on the geographical location where it was produced, some buckwheat could be an excellent source of dietary selenium.

B. Other significant accomplishments:
Animal studies are needed to confirm the value of buckwheat as a dietary source of minerals, however diets containing buckwheat have not been developed. Diets that included buckwheat as a major ingredient were designed and tested at the GFHNRC; because raw buckwheat contains anti-nutritional components, the buckwheat was cooked, dried and powdered before inclusion in the diet. It was demonstrated that diets containing 20% buckwheat flour maintained the same growth rate as the standard AIN-93 experimental diet. Development of these diets will allow studies of the nutritional value of buckwheat to be conducted under controlled conditions.

C. Special populations:
None

D. Progress Report
This report serves to document research conducted under a Trust Fund Cooperative Agreement, #58-5450-3-406, between ARS and MinDak Growers, Ltd. Additional details of the research can be found in the report for parent CRIS 5450-51000-032-00D.

The purpose of this agreement is to study the nutritional value of buckwheat, with emphasis on buckwheat as a source of nutritionally essential minerals. MinDak Growers, Ltd. is collecting buckwheat and soil samples from buckwheat producers, and preparing those samples for analysis. The GFHNRC is conducting all chemical analyses, and is planning and conducting studies in rats to determine the bioavailability of minerals from buckwheat.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

Project Number: 5450-51000-033-01T **Accession:** 0403544 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: DETERMINATION OF A NO EFFECT LEVEL FOR COPPER IN DRINKING WATER FOR FUTURE DIETARY SUPPLEMENT STUDY

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences. This is of concern because low dietary intakes of copper have been associated with the incidence of some chronic diseases such as ischemic heart disease and osteoporosis. Drinking water can sometimes contribute to total copper intake, but this contribution may be limited by regulatory groups attempting to assure quality potable drinking water, in part, by setting low concentration standards for copper. Human volunteers were used to identify the chemical forms and doses of copper most useful in dietary supplementation trials and to measure the concentrations of copper in drinking water intolerable to healthy adults.

2. **How serious is the problem? Why does it matter?**
Some nutritional scientists believe that the best method of defining the nutritional requirement for copper is to conduct dietary supplementation trials. Cupric oxide, a chemical form of copper in multinutrient supplements, recently has been found to be relatively unabsorbable. Supplements with improved absorbability may cause undesirable acute effects such as transient nausea. Moreover, environmental regulatory agencies are reevaluating the quality of drinking water and may decrease the amount of copper because of these undesirable effects that will mandate a change in water purification processes. This research will help establish the safe and tolerable dose of copper as an absorbable supplement and in drinking water. This work is relevant to the Food and Drug Administration, the Environmental Protection Agency, nutritionists, physicians, researchers on heart disease and osteoporosis and various local and state agencies that must respond to federal standards on water quality.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
The research fits into the National Program 107 Human Nutrition and the Performance Goal 3.1.1. Human Nutrition Requirements. Emphasis is on biomarkers, mechanisms of action and environmental factors.

4. **What were the most significant accomplishments this past year?**
This report serves to document research conducted under a Trust Fund Cooperative Agreement between the ARS and the International Copper Association, Inc. Additional details of research can be found in the report for the paretn project 5450-51000-033-00D.

The tolerable concentration of copper in drinking water is unknown. Men and women drank mineral water varying in copper sulfate content and answered questionnaires about symptoms and responses at the Grand Forks Human Nutrition Research Center and at the Universities of Ulster in Northern Ireland and Santiago in Chile, and in Shanghai, China. The copper threshold for transient nausea (taste being rather

Project Number: 5450-51000-033-01T **Accession:** 0403544 **FY:** 2003

insensitive) is approximately 5 mg/1. Because this concentration exceeds the U.S. regulatory level of 1.3 mg/l, the Environmental Protection Agency need not change the regulations.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
A similar project done earlier revealed the threshold for copper in distilled water to be 4 mg/l; the data have been published in Regulatory Toxicology and Pharmacology.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
A manuscript based on the results of the human study (Question 4) has been written on the different amounts of copper sulfate in mineral water; it will be submitted for publication. We will evaluate the usefulness of these data for providing nutritional supplements to people of low copper status. In 2004 we will publish these data and terminate the project.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
None.

Publications: Log 115:

1. Poirier, K.A., Chen, B.H., Zhu, H.G., Shi, W., Araya, M., Johnson, L., Klevay, L., Nielsen, F., Robson, P., Strain, S., Baker, S. The determination of human acute no observed adverse effect level (NOAEL) for copper: Effects of volume and dose. Society for Risk Analysis 2001. Risk Analysis in an Interconnected World. Final Program. 2001. Abstract p. 81. Presented by Ken Poirier at the meeting of the Society for Risk Analysis 0000123926

2. Poirier, K.A., Araya, M., Klevay, L.M., Nielsen, F.H., Johnson, L., Strain, J.J., Robson, P., Chen, B.H., Wei, S., Zhu, H.G., Baker, S.R. Determination of a human acute no-observed-adverse-effect level (NOAEL) for copper in bottled drinking water: Volume, dose and concentration considerations. The Toxicologist. 2002. v. 66. p. 102. Presented by Ken Poirier at Society of Toxicology meeting. 0000127293

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-034-01T **Accession:** 0401535 **FY:** 2003
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENTS FOR OPTIMAL FUNCTION AND HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OFSERINE PROTEASES

Period Covered From: 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
The cause of rheumatoid arthritis is unknown, a cure is unavailable, and the lifetime cost of rheumatoid arthritis for an individual patient can be as much as $250,000. Thus, research is needed to find the cause of the disease and more effective treatments. Excessive inflammation leads to inflammatory disease (for example, rheumatoid arthritis). Certain boron compounds are potent in vitro inhibitors of several enzymes that regulate the normal inflammatory reaction. Therefore, the focus of this project is to identify inflammatory mediators that interact with boron. This approach will help establish the specific function of boron in humans and how dietary boron influences rheumatoid arthritis. Based on molecular structure, several classes of compounds predicted to interact with boron will be selected and assessed to determine the exact role of boron in regulation of the inflammatory response. The basic approach will be to determine the in vitro binding affinities of these compounds to boron. Subsequently, compounds with physiologically significant boron binding affinities will be investigated as potential indicators of boron status by in vivo measurement of these compounds after extraction from tissues of animals fed boron deficient diets.

2. **How serious is the problem? Why does it matter?**
Inflammatory diseases cost Americans billions of dollars yearly in treatment and loss of productivity. Rheumatoid arthritis in particular is a painful, chronic, recurrent, systemic inflammatory disease that affects 1 3 percent of Americans. Thus, prevention or significant amelioration of inflammatory diseases including rheumatoid arthritis by relatively simple dietary means, would have significant impact. There is a high probability that normal amounts of dietary boron will significantly ameliorate symptoms of rheumatoid arthritis in humans based on its known effect on cartilage development and maintenance and influence on the progression of experimental rheumatoid arthritis in animal model systems. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
The research relates to the National Program 107, Human Nutrition (100%), specifically, the National Program Component of Human Nutrition Requirements: "determine requirements for nutrients and other food components of children, pregnant and lactating women, adults, and elderly of diverse racial and ethnic backgrounds." The research relates to the "Mechanism of Action" objective within this component: "Identify and fully characterize mechanisms of action for beneficial effects of known nutrients and other potentially beneficial dietary chemicals; measure the size of the effects associated with specific amounts of the chemical or nutrient component in question."

Project Number: 5450-51000-034-01T **Accession:** 0401535 **FY:** 2003

4. **What were the most significant accomplishments this past year?**
A. The research phase of this project is complete and manuscripts are being prepared. Therefore there are no new accomplishments to report for this year.

D. This report serves to document research conducted under Trust Fund Cooperative Agreement #58-5450-8-116 between ARS and U.S. Borax, Inc. Additional details of research can be found in the report for the parent project 5450-51000-034-00D Mineral Intakes for Optimal Bone and Joint Development and Health.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
An in vitro model system was developed and implemented to determine the direct binding of boron to biomolecules. The method utilizes capillary electrophoresis technology and allows for the discrete separation and identification of biomolecules that bind to boron and also allows for discrimination of binding interactions. When molecules are separated by capillary electrophoresis in the absence or presence of boron, the degree of boron binding is indicated as a change in migration time of the molecule through the solution. An increase in migration time indicates increased boron binding. Various biomolecules can be compared for their ability to bind boron. The information may be used to determine the importance the exact biological role of boron in humans and the importance of that role in the inflammatory response.

The new capillary electrophoresis method described above was used to demonstrate the direct binding of boron to biomolecules of considerable physiological importance. Normal physiological amounts of boron were found to bind to S-adenosylmethionine, the predominant methyl donor in biological methylations and a versatile co-factor in a variety of physiological processes. These findings suggest that boron, a natural dietary component, may be important in the regulation of substrate methylation in biochemical pathways.

A new methodology that uses flow cytometry was developed to accurately determine the amount of media taken up by human cells cultured in growth media.

6. **What do you expect to accomplish, year by year, over the next 3 years?**
None.

7. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None.

8. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).**
None.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-01S **Accession:** 0401877 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: SELENIUM IN CONTENT OF BEEF AND WHEAT

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
None.

2. **How serious is the problem? Why does it matter?**
None.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
None.

4. **What were the most significant accomplishments this past year?**
This report serves to document research conducted under a Specific Cooperative Agreement between ARS and North Dakota State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

This was part of a series of studies that have examined the production of beef in Se-enriched areas of North and South Dakota. This project is distinct from project 5450-51000-032-06S (NDSU Agricultural production Aspects of High-Se Meat) in that this project focuses on determining whether high-Se meat produced in the Dakota¿s can be turned into a marketable product, whereas project 06S is concerned with the overall production Animal Science aspects of using high-Se feedstuffs in cattle rations.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/15/2003

Project Number: 5450-51000-032-03T **Accession:** 0402749 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: HIGH SELENIUM BEEF PRODUCED IN NORTH DAKOTA

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**

2. **How serious is the problem? Why does it matter?**

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2003: This agreement is being terminated this year. There are no new accomplishments to report.

D. Progress Report: This report serves to document research conducted under Trust Fund Cooperative Agreement #58-5450-8-113 between ARS and the North Dakota Beef Commission. Additional details of the research can be found in the report for the parent CRIS 5450-51000-032-00D.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003

Project Number: 5450-51000-032-12S **Accession:** 0401621 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS IN RE-SEARCH ON MINERAL REQUIREMENTS IN HUMAN NUTRITION

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. None.

B. None.

C. None.

D. This report serves to document research conducted under Specific Cooperative Agreement #58-5450-8-111 between ARS and University of North Dakota Physics Department (Dr. Glenn Lykken, Health Physicist). Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-032-00D and related CRIS project 5450-51000-034-00D.

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true ¿tracer¿ that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, applying an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

Accomplishments included health physicist support of investigations to assess bioavailability of calcium, iron, manganese and zinc using gamma-emitting radiotracers in human absorption studies. The health physicist led the work of the FDA-sanctioned UND Radioactive Drug Research Committee, which reviewed and provided oversight for all nutrition experiments employing radioactive tracers. A HpGe detector was established at the gamma spectroscopy laboratory in the UND Physics Department, which allows accurate assay of radioisotopes for gamma emitting impurities. This detector was applied to help verify the gamma-emitting spectra of Ca-47 and of an Fe-55 elemental iron fortification powder that had been produced by neutron-activation. Successful radiotracer experiments with Ca-47 included an evaluation of photo peaks of Sc-47, for possible extension of the monitoring of Ca-47 retention. Gamma ray background radiation in the steel room of the whole body

Project Number: 5450-51000-032-12S **Accession:** 0401621 **FY:** 2003

counter was investigated by studying the effect of airflow on variations in radiation, detected by a sealed reference detector. A mathematical model to correct whole body counting data for individual body sizes (using a family of anthropomorphic "broad-beam" planar radiation sources, Four-Gamma Anthropomorphic Uniform Isotope source (4GAU) capable of emitting four gamma rays over the energy range 122 to 1274 keV) was developed, and continuing work on such algorithms will result in more accurate measurements of total body potassium as well as the requirements and metabolism for mineral elements including copper, calcium, iron, manganese, selenium and zinc. These accomplishments support the sensitive detection of gamma-emitting isotopic tracers, providing uniquely sensitive measurements of mineral retention in humans, as affected by nutritional status, dietary sources of nutrients, and the human genotype.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/10/2003

Project Number: 5450-51000-032-15T **Accession:** 0403340 **FY:** 2003
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MINERAL NUTRIENT INTAKE & MECHANISMS OF UTILIZATION FOR HEALTH
NPL Leader: KATHLEEN C ELLWOOD

National Programs: 107 100% Human Nutrition

Title: CORRELATION BETWEEN SPERM MOTILITY AND HEAVY METALSTATUS OF ANIMALS AND HUMANS

Period Covered **From:** 10/2002 **To:** 9/2003 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it?**
In the equine breeding industry, cryopreservation of the semen has not been perfected to the point where the freeze-thaw cycle does not cause low viability and sperm count, and/or low sperm motility. This results in reduced ability of the sperm to fertilize the egg. Human male fertility studies have shown that the trace element content of the semen, especially zinc, is an important factor that helps control sperm count, viability, and motility. We question whether this could be part of the cause, if not the sole cause, of similar problems in horses. In addition, could other trace elements at either essential and/or toxic concentrations, be part of the causative factor?
The initial phase of this study will be to collect body fluids such as semen, plasma, and urine from a population of stallions and measure the trace element content to ascertain the physiological status of each element. Human samples also will be collected. We will determine if there is a correlation between low to high concentrations of the elements and sperm viability, motility, and survival time during cryopreservation. The trace elements to be analyzed include calcium, zinc, copper, iron, selenium, cadmium, chromium, and others that might be of interest.

2. **How serious is the problem? Why does it matter?**
As new reproductive technologies have become available to the horse breeding industry, it has become apparent that there are unexpected problems affecting semen preservation and sperm viability. At present there is no good way to ship frozen semen to maintain high sperm viability, thus causing a major loss of the product at great cost to the breeders. Some of these problems could be related to the mineral content of the semen. There are viability problems with human sperm as well that could have a similar etiology.

3. **How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?**
This research will develop information about the effects of various mineral elements on the viability, motility, and biochemical functions of animal and human sperm. In addition, this research will allow us to determine safe and adequate intakes of mineral elements for optimal health and bodily functions. Thus, the research relates to National Program 107, Human Nutrition (100%) and the nutrient requirements component of the program. Priority objectives addressed by the research are biomarkers, mechanism of action, nutrient interactions, environmental factors, and function and performance.

4. **What were the most significant accomplishments this past year?**
A. Single Most Significant Accomplishment during FY2001:
None. The status of this project has not changed from last year.

B. Other Significant Accomplishments:
None.

Project Number: 5450-51000-032-15T **Accession:** 0403340 **FY:** 2003

C. Significant Activities that Support Special Target Populations.
None.

D. Progress Report.
Because of the proprietary nature of the data gathered in this project, the collaborators on the Trust Agreement with HR Enterprises (CancerOne) of Moscow, ID, have treated the results of the project as confidential. The project is closed.

Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/22/2003